AD_____

Award Number: Contract No. W81XWH-11-1-0626

TITLE: Treatment of Fragile X Syndrome with a Neuroactive Steroid

PRINCIPAL INVESTIGATOR: Randi Hagerman, M.D.

CONTRACTING ORGANIZATION: University of California, Davis
Sacramento, CA 95817

REPORT DATE: August 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2014	Annual	15 Jul 2013 – 14 Jul 2014

4. TITLE AND SUBTITLE
Treatment of Fragile X Syndrome with a Neuroactive Steroid

5a. CONTRACT NUMBER

5b. GRANT NUMBER W81XWH-11-1-0626

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Randi Hagerman, M.D.

E-Mail: randi.hagerman@ucdmc.ucdavis.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)

University of California, Davis
Sacramento, CA 95817

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
We renewed the protocol with the UC Davis (UCD) IRB and the USAMRMC HRPO. This past year (Jul 15, 2013 to Jul 14, 2014), twenty-five subjects were enrolled, bringing total enrollment to thirty-six. Twenty-six have completed and there have been seven early terminations and two screen failures. Data-entry is done regularly. No serious adverse events have occurred. The first DSMB meeting took place in September 2013, and the trial was allowed to proceed. We plan to increase recruitment to 4 subjects per month for Year 4 of the project to meet the enrollment goal of sixty. If we can demonstrate efficacy of ganaxolone in our outcome measures this will have a major impact on the treatment of fragile X syndrome and move us closer to a cure for these children.

15. SUBJECT TERMS
Nothing Listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	47	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

INTRODUCTION

This study is a Phase II trial to assess the safety, tolerability, and efficacy of ganaxolone, a $GABA_A$ agonist, for the treatment of behavioral problems including anxiety and inattention in children with FXS. It has been demonstrated in the fragile X mouse model and the Drosophila (fruit fly) model of FXS that the $GABA_A$ system including multiple receptors is dramatically down-regulated. Ganaxolone is a drug that enhances $GABA_A$ activity. We hypothesized that ganaxolone will significantly improve behavioral problems including anxiety, inattention and impulsivity problems in children with fragile X syndrome. We will enroll 60 children, ages 6-17 years, with fragile X syndrome over a 4-year period and they will be randomized to receive either ganaxolone or a placebo initially and then crossed over after 6 weeks. We will use innovative outcome measures in addition to standard outcome measures that have been successful in previous treatment trials in fragile X syndrome at baseline and follow-up visits.

BODY

TASKS 1, 2 and 3 were completed in the beginning of Year 2. Regarding TASK 4, we are actively recruiting subjects at a rate of 3-4 individuals per month to meet the enrollment goal of sixty. From Jul 15, 2013 to Jul 14, 2014, twenty-five subjects were enrolled, screened and randomized, bringing the total enrollment to thirty-six. Twenty-six have completed and there have been seven early terminations and two screen failures. Data-entry is being completed on a regular basis. No serious adverse events have occurred. The first Data Safety Monitoring Board (DSMB) meeting took place in September 2013, and the trial was allowed to proceed. The next DSMB meeting is being scheduled for Fall 2014. We plan to increase recruitment to 4 subjects per month for Year 4 of the project to reach the enrollment goal of sixty.

Work on TASK 5, data-analysis and report writing, has not begun. We will begin working on this task in the second half of Year 4.

KEY RESEARCH ACCOMPLISHMENTS

There are no key research accomplishments to report since the trial is still actively recruiting and data analysis has not been completed.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

1. R. Lozano, E. Hare, R. Hagerman, *Neuropsychiatric Disease and Treatment* **10**, 1769 (2014).
2. E. Hare, R. Hagerman, R. Lozano, *Expert Opinion on Orphan Drugs,* **2**, 531 (2014).
3. R. Lozano, E. Hare, R. J. Hagerman, in *Treatment of Neurodevelopmental Disorders Targeting Neurobiological Mechanisms,* R. J. Hagerman, R. L. Hendren, Ed. (Oxford University Press, 2014), pp. 215-238.

CONCLUSION

The protocol was renewed with both the UC Davis (UCD) IRB and the USAMRMC HRPO. Since enrollment began in November 2012, thirty-six subjects have been enrolled with twenty-six completed. There have been seven early terminations and two screen failures. Data-entry is done regularly. No serious adverse events have occurred. The first DSMB meeting took place in September 2013, and the trial was allowed to proceed. The next DSMB meeting will be scheduled for Fall 2014. We plan to increase recruitment to 4 subjects per month for Year 4 to reach the enrollment goal of sixty. If we can demonstrate efficacy of ganaxolone in our outcome measures this will have a major impact on the treatment of fragile X syndrome and move us closer to a cure for these children.

REFERENCES: List all references pertinent to the report using a standard journal format (i.e. format used in *Science, Military Medicine,* etc.).

1. R. Lozano, E. Hare, R. Hagerman, *Neuropsychiatric Disease and Treatment* **10**, 1769 (2014).
2. E. Hare, R. Hagerman, R. Lozano, *Expert Opinion on Orphan Drugs,* **2**, 531 (2014).
3. R. Lozano, E. Hare, R. J. Hagerman, in *Treatment of Neurodevelopmental Disorders Targeting Neurobiological Mechanisms,* R. J. Hagerman, R. L. Hendren, Ed. (Oxford University Press, 2014), pp. 215-238.

APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

1. R. Lozano, E. Hare, R. Hagerman, *Neuropsychiatric Disease and Treatment* **10**, 1769 (2014).
2. E. Hare, R. Hagerman, R. Lozano, *Expert Opinion on Orphan Drugs,* **2**, 531 (2014).
3. R. Lozano, E. Hare, R. J. Hagerman, in *Treatment of Neurodevelopmental Disorders Targeting Neurobiological Mechanisms,* R. J. Hagerman, R. L. Hendren, Ed. (Oxford University Press, 2014), pp. 215-238.

Neuropsychiatric Disease and Treatment

Dovepress

open access to scientific and medical research

Open Access Full Text Article

REVIEW

Modulation of the GABAergic pathway for the treatment of fragile X syndrome

This article was published in the following Dove Press journal:
Neuropsychiatric Disease and Treatment
16 September 2014
Number of times this article has been viewed

Reymundo Lozano[1,2]
Emma B Hare[1,2]
Randi J Hagerman[1,2]

[1]MIND Institute, [2]Department of Pediatrics, UC Davis Medical Center, Sacramento, CA, USA

Abstract: Fragile X syndrome (FXS) is the most common genetic cause of intellectual disability and the most common single-gene cause of autism. It is caused by mutations on the fragile X mental retardation gene (*FMR1*) and lack of fragile X mental retardation protein, which in turn, leads to decreased inhibition of translation of many synaptic proteins. The metabotropic glutamate receptor (mGluR) hypothesis states that the neurological deficits in individuals with FXS are due mainly to downstream consequences of overstimulation of the mGluR pathway. The main efforts have focused on mGluR5 targeted treatments; however, investigation on the gamma-aminobutyric acid (GABA) system and its potential as a targeted treatment is less emphasized. The fragile X mouse models (*Fmr1*-knock out) show decreased GABA subunit receptors, decreased synthesis of GABA, increased catabolism of GABA, and overall decreased GABAergic input in many regions of the brain. Consequences of the reduced GABAergic input in FXS include oversensitivity to sensory stimuli, seizures, and anxiety. Deficits in the GABA receptors in different regions of the brain are associated with behavioral and attentional processing deficits linked to anxiety and autistic behaviors. The understanding of the neurobiology of FXS has led to the development of targeted treatments for the core behavioral features of FXS, which include social deficits, inattention, and anxiety. These symptoms are also observed in individuals with autism and other neurodevelopmental disorders, therefore the targeted treatments for FXS are leading the way in the treatment of other neurodevelopmental syndromes and autism. The GABAergic system in FXS represents a target for new treatments. Herein, we discuss the animal and human trials of GABAergic treatment in FXS. Arbaclofen and ganaxolone have been used in individuals with FXS. Other potential GABAergic treatments, such as riluzole, gaboxadol, tiagabine, and vigabatrin, will be also discussed. Further studies are needed to determine the safety and efficacy of GABAergic treatments for FXS.

Keywords: gamma-aminobutyric acid (GABA) system, targeted treatments, autism, ganaxolone, arbaclofen

Introduction

Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability and largest single-gene cause of autism.[1] The fragile X mental retardation gene (*FMR1*) located on the X chromosome at band q27.3 typically has 5–44 CGG repeats; however, this trinucleotide repeat length can expand to an unstable repeat length. 45-54 CGG repeats is considered a gray zone and may have some clinical involvement, including a higher rate of primary ovarian insufficiency compared to controls.[2] Premutation carriers have trinucleotide repeats ranging from 55–200 CGG in length. These are usually healthy individuals who are at risk of

Correspondence: Reymundo Lozano
MIND Institute, UC Davis Medical Center, 2825 50th Street, Sacramento, CA 95817, USA
Tel +1 916 703 0494
Email reymundo.lozano@ucdmc.ucdavis.edu

developing fragile X-associated tremor/ataxia syndrome, a neurodegenerative disorder seen in aging carriers.[3] They are also at an increased risk for anxiety and mood disorders,[4] immune-mediated disorders,[5] migraine headaches,[6] hypertension,[7] and primary ovarian insufficiency,[8] all of which range in severity and prevalence. Adult female carriers have evidence of gamma-aminobutyric acid (GABA) dysfunction.[9]

There are some carriers who may show an attention-deficit hyperactivity disorder (ADHD) and autism spectrum disorders (ASDs);[10] however, the prevalence of those traits is far more common in individuals with the full mutation whose trinucleotide expansion is greater than 200 CGG repeats. The full mutation presents with a range of clinical features, including ADHD, ASDs, anxiety, intellectual disability, social avoidance, aggression, stereotyped behaviors, disrupted sleep patterns, epilepsy, macroorchidism, prominent ears, long faces, soft skin, and hyperextensible joints.[11] Males with the full mutation are typically more severely affected than females due to the compensatory nature of the second X chromosome, which still translates the *FMR1* gene leading to more normal fragile X mental retardation protein (FMRP) production. The prevalence of the full mutation is approximately one in 5,000 males[12] and one in 8,000 females,[13] whereas the premutation is far more common, at one in 130–250 females and one in 250–810 males.[14]

The expansion of the trinucleotide sequence results in lowered FMRP levels. The premutation expansion results in a two- to eightfold increase in *FMR1* mRNA levels, which leads to RNA toxicity accounting for phenotypic features; however, the FMRP levels often stay normal or become somewhat decreased, particularly after 120 CGG repeats.[15,16] The full mutation results in reduced or no FMRP production, which is found primarily in the brain and testes. In the brain, FMRP is crucial to synaptic development and refinement thus accounting for the psychiatric features of FXS, while its presence in the testes is needed for normal testicular size. The primary function of FMRP is to negatively regulate protein translation at the synapse,[17] and it is estimated that FMRP binds up to 4%–8% of mRNA within the brain.[18] This regulation is crucial in the metabotropic glutamate receptors (mGluRs) 1 and 5, which become upregulated, leading to exaggerated long-term depression (LTD). This concept is the basis of the mGluR theory of FXS[19] and the focus of many targeted treatments for FXS, such as the mGluR5 antagonists AFQ056 (mavoglurant; Novartis Pharmaceuticals, Basel, Switzerland) and RO4917523 (F Hoffmann-La Roche AG, Basel, Switzerland).

Additionally implicated in the dysregulation at the synapse is the GABA system, which is important in synaptic inhibition. An imbalance between the excitatory glutamatergic and the inhibitory GABAergic neurotransmission is proposed to cause the cognitive impartments, anxiety, and autism of FXS and other neuropsychiatric and neurodevelopmental disorders.[1,20,21] While two medications have been developed specifically for GABAergic modulation – arbaclofen (Seaside Therapeutics, Cambridge, MA, USA) and ganaxolone (Marinus Pharmaceuticals, Inc., New Haven, CT, USA) – this paper will also address GABAergic medications such as riluzole, gaboxadol, tiagabine, and vigabatrin. Before discussing the medications, a more in-depth focus on the GABA system and its dysregulation in FXS will follow.

Neurobiology

More than 3 decades of molecular research have led to a better understanding of the neurobiology of FXS and related disorders. FXS is caused by a dynamic mutation of more than 200 CGG trinucleotide repeats in the 5' untranslated region on the *FMR1* gene, which results in an absence of expression of FMRP. FMRP is a selective RNA-binding protein, found most abundantly in the CNS and testes, which regulates the expression of many mRNAs through inhibitory control at the synapse.[22] The crucial role of FMRP in regulating the synthesis of synaptic proteins extends beyond the phenotype of FXS to other neurodevelopmental and neuropsychiatric disorders, in which FMRP may also be deficient.[23]

FMRP contains three main RNA-binding domains: two hnRNP K-homology domains and one arginine- and glycine-rich region of FMRP. In vitro FMRP is part of messenger ribonucleoparticles (structures that are involved in protein synthesis) and regulates dendritic transport of associated mRNAs.[22] FMRP interacts with several cytoplasmic and nuclear proteins and has been found in granules containing translationally silent preinitiation complexes. In summary, FMRP regulates RNA transportation, stabilization, and translation, mainly at the synapse in neurons.

The activation of mGluR5 induces protein synthesis in the soma, axons, dendrites, and postsynaptic sites, as well as degradation and recycling of somatic and axonic proteins through the MAPK/ERK and mTOR pathways, which is required for LTD, a form of hippocampal synaptic plasticity that develops and consolidates long-term memories. In the

Fmr1-knock out [KO] mice, LTD is significantly increased, and this leads to deficits in synaptic plasticity and weakening of synaptic connections.[22] The mGluR theory, which suggests that psychiatric and neurological aspects of the syndrome are due to exaggerated downstream consequences of mGluR5 upregulation, was validated by genetic mouse studies in which rescue of several symptoms occurred when the *Fmr1*-KO mouse was crossed with the mGluR heterozygous mouse.[24] The *Fmr1*-KO also shows hypothalamic excess of many synaptic proteins from increased protein translation rate and protein synthesis, whereas the GABA system is downregulated in the absence of FMRP, as described in the GABA neurobiology section.[25]

GABA neurobiology

The GABA system is the main inhibitory system in the brain. It works through two classes of GABA receptors: $GABA_A$ and $GABA_B$. $GABA_A$ receptors are ligand-gated ion channels, whereas $GABA_B$ receptors are G protein-coupled receptors. $GABA_A$ receptors allow the flow of chloride ions across the membrane, which hyperpolarizes the neuron's postsynaptic membrane and minimizes the effect of any coincident synaptic input. $GABA_B$ receptors hyperpolarize the neuron's membrane by activating G-protein-coupled inwardly rectifying potassium channels.[26] The GABA receptors are very diverse in their subunit composition and localization at the synapse and in regions of the brain; their effects are fast or slow persistent-tonic inhibition, depending on their localization and grade of stimulation.[27]

The GABA system is required for a balanced neuronal activation and network oscillations, direct flow of information, neural synchrony, and facilitating the movement of information in and between multiple brain areas involved in cognition.[28–30] Since FXS is characterized by anxiety, hyperarousal, and epilepsy,[31,32] recent studies[33–36] have aimed to identify the defects in the inhibitory GABA system in the *Fmr1*-KO mouse.

$GABA_A$

FMRP targets the mRNAs encoding eight different $GABA_A$ receptor subunits ($\alpha1$, $\alpha3$, $\alpha4$, $\beta1$, $\beta2$, $\gamma1$, $\gamma2$, and δ), which were significantly reduced in the neocortex and cerebellum of *Fmr1*-KO mice, particularly the γ subunit, which represents extrasynaptic (perisynaptic) $GABA_A$ receptors.[37,38] The *Fmr1*-KO mouse confirms a deficit in the production of $GABA_A$ receptors. Proteins required for GABA transport (eg, GABA transporter) and catabolism (eg, GABA transaminase [GABA-T], succinic semialdehyde [SSADH]) are also reduced.[33,34] In addition, FMRP in the presynaptic side also regulates the expression of glutamic acid decarboxylase, which is the rate-limiting GABA-synthesis enzyme.[33,35,37] Therefore, FMRP regulates the expression, metabolism, and catabolism of the $GABA_A$ receptors, and in its absence, there is a decreased GABAergic input. The *Fmr1*-KO mouse exhibits reduced inhibitory postsynaptic currents in the amygdala[35] and subicular neurons.[36] Although FMRP inhibits the translation of many messages, it can also stimulate the translation of mRNAs, and the GABA system is downregulated in the absence of FMRP. Consequences of the reduced $GABA_A$ receptor expression in FXS likely include oversensitivity to sensory stimuli, seizures, and anxiety.

$GABA_B$

$GABA_B$ is a metabotropic G protein-coupled receptor that regulates voltage-gated Ca^{2+} channels, G protein inwardly rectifying K^+ channels, and adenylyl cyclase.[26] Activation of the receptor triggers slow inhibitory postsynaptic currents, which reduce the neuronal excitability.[26] Presynaptic $GABA_B$ receptors inhibit glutamate release, which further exacerbates the upregulation of the mGluR5 system; therefore, treatment with $GABA_B$ modulators has the potential to correct phenotypic deficits in FXS. When treated with a double-knockout of *FMR1* and *RGS4*, male *Fmr1*-KO mice showed reduced susceptibility to audiogenic seizures.[39] Since *RGS4* is a regulator of G protein signaling and associated with $GABA_B$ receptors and inward-rectifying K^+ channels, it has a therapeutic potential to regulate $GABA_B$ subunits in the treatment of FXS.[39]

Impacted regions of the brain

The amygdala, a component of the limbic system involved in emotional recognition and reaction, as well as fear processing, is one part of the brain considerably impacted by GABAergic dysregulation. Socioemotional impairments are prevalent in both premutation and full mutation patients, which warrants study of the amygdala as a basis for this impairment. Numerous studies have been performed assessing amygdala volume, activation in relation to gaze processing, and facial-emotional processing, which yield contradictory and inconsistent results due to varied protocols and populations (as summarized in Kim et al).[40] Fear-specific activation of the amygdala was significantly reduced in patients with FXS compared to neurotypical controls, despite no differences in amygdala volume,[40] which suggests biological impairment possibly at the synaptic level instead of altered brain size leading to emotional dysregulation. The amygdala's phasic

inhibitory postsynaptic currents, tonic inhibitory currents, reduced GABA release, and inhibitory synapses are considerably reduced in frequency and amplitude in the amygdala of *Fmr1*-KO mice.[35] Due to the phenotypic socioemotional impairments seen in both the premutation and full mutation individuals alongside the biological impairments seen in the *Fmr1*-KO mouse, the amygdala is a point of study for gauging improvement with new treatments.

Further modifications in GABAergic activity are found in the cortex of the brain in FXS and, while monosynaptic GABAergic transmission is unaffected, there is a substantial deficit in local excitatory drive targeting fast-spiking inhibitory neurons in layer 4 of the somatosensory (barrel) cortex, which partially accounts for seizures, cognitive dysfunction, and sensory hypersensitivity of the FXS phenotype.[41]

A study assessing GABA-mediated cerebellar inhibition comparing healthy, asymptomatic women with and without the premutation found that women with the premutation show an absence of cerebellar inhibition over primary motor cortex as well as reduced $GABA_A$-mediated intracortical and afferent inhibition.[9] Even in asymptomatic carriers, there is still GABA dysregulation, which warrants the need for compounds with GABAergic modulation properties in premutation carriers. The available compounds are limited and mostly focus on antiepileptic properties; however, other mechanisms of action modified by such treatments may provide additional outlets for improving the phenotype in both carriers and individuals affected by the full mutation.

GABAergic treatments

After a better understanding of the neurobiology and neuropathogenesis of FXS, many compounds have been used as targeted treatments for FXS. Research has focused mainly on the development of mGluR5 antagonists, but better understanding of the GABAergic system in FXS has led to a new GABAergic approach and relevant targeted treatment. The GABA receptors in different regions of the brain are associated with some behavioral phenotypes in individuals with FXS. Particular attention has been devoted to correcting the amygdala-based symptoms. GABAergic agonists can be used for specific phenotypes including anxiety, autistic behavior, epilepsy, and cognitive impairment. GABA agonists have shown very limited efficacy in preliminary studies for these symptoms, but they have been well tolerated.[42,43] The GABAergic treatments are a relatively new area and basic and translational research is limited in spite of their potential to treat FXS based on animal and small clinical studies.

Acamprosate

Acamprosate is a $GABA_A$ agonist approved for treatment of alcohol withdrawal. Excessive alcohol consumption over a long period of time changes the balance between the excitatory and inhibitory systems. Acamprosate helps people who have consumed large amounts of alcohol by stabilizing the excitatory/inhibitory balance in the brain, mainly by enhancing the function of $GABA_A$ receptors and, possibly, by its inhibitory effects on the mGluRs.[44] Acamprosate does not prevent the withdrawal symptoms that people may experience when they stop drinking alcohol.

Clinically, a short report of three patients with FXS treated with acamprosate showed improvements in language and behavior,[44] which led to other GABAergic targeted treatments and a second open-label 10-week trial of acamprosate (mean dose 1,054±422 mg/day) in 12 children and adolescents ages 6 to 17 years with FXS. The study showed improvements in social behavior and inattention/hyperactivity using multiple standard behavioral outcome measures. A Clinical Global Impressions-Improvement (CGI-I) scale score of "very much improved" or "much improved" was rated in nine of 12 (75%) subjects. No significant adverse effects were reported. Additionally, pre- and posttreatment blood biomarker analyses were performed by measuring brain-derived neurotrophic factor (BDNF) levels. A significant increase in the BDNF levels with treatment was described. However, treatment response did not correlate with change in the BDNF level.[44]

Ganaxolone

Ganaxolone (3a-hydroxy-3B-methyl analog of allopregnanolone) is a $GABA_A$ receptor agonist through allosteric modulation that has anticonvulsant, anxiolytic, and sedative effects.[45] It is orally active and does not have hormonal effects. Neuroactive steroids like ganaxolone act most potently and effectively on $GABA_A$ receptors containing δ subunits. Ganaxolone is under development for treatment of seizure disorders and posttraumatic stress disorder. This pharmaceutical has been well tolerated, is safe in adults, children, and infants,[46] and has been found to improve symptoms in humans and mouse models.[46,47]

Although many $GABA_A$ receptor subtypes are diminished in the *Fmr1*-KO mouse, there is evidence that extrasynaptic γ subunit containing $GABA_A$ receptors are especially affected.[48] In the *Fmr1*-KO, ganaxolone has been shown to decrease

audiogenic seizures.[47] Similarly, studies in the *dfmr* mutant fly showed that $GABA_A$ agonists ameliorate the lethality phenotype of glutamate-containing food, neuropathology, excessive protein translation, and abnormal courtship behavior.[49] The most frequently reported adverse events with ganaxolone in seizure studies are somnolence, convulsion, agitation, pharyngitis, otitis media, diarrhea, vomiting, cough, and pyrexia.[50] A randomized, Phase II, double-blind, placebo-controlled crossover trial to investigate the efficacy of ganaxolone for the treatment of anxiety and attention deficits in children with FXS aged 6 to 17 years (http://www.ClinicalTrials.gov; NCT01725152)[51] is currently under way. Ganaxolone should increase and normalize $GABA_A$-mediated signaling – by boosting the signaling capacity of existing receptors – and improve behavior, particularly anxiety and attention.

Gaboxadol (THIP)

Tonic $GABA_A$ inhibition is associated with specific receptor subunits, particularly the relatively rare α4, α6, and δ subunits. The δ subunit-containing receptors are insensitive to benzodiazepine agonists,[52,53] but highly sensitive to gaboxadol.[54,55] Gaboxadol, the selective δ-$GABA_A$ super-agonist receptor, 4,5,6,7-tetrahydroisoxazolo(5,4-c) pyridin-3-ol (also known as THIP), has properties to generate tonic inhibition, namely activation by low concentrations of GABA through the activation of the extrasynaptic δ subunit-containing receptors.[54,56]

As previously mentioned, *Fmr1*-KO mouse studies have showed deficits in inhibitory transmission in the amygdala of the *Fmr1*-KO mouse, including reduction in the frequency and amplitude of phasic inhibitory postsynaptic currents and of tonic inhibitory currents, as well as a reduction in the number of inhibitory synapses and in neuronal hyperexcitability in principal neurons.[35]

A neuronal study has also shown significant increases in the action potential threshold in both wild-type and *Fmr1*-KO mice.[57] Strikingly, the action potential threshold in *Fmr1*-KO mice in amygdala slices was restored to wild-type levels by THIP application. Thus, the electrophysiological abnormalities of neuronal hyperexcitability in the *Fmr1*-KO amygdala can be dramatically rescued by augmenting tonic inhibitory tone.[35] A behavioral study in *Fmr1*-KO mice have shown that THIP significantly attenuated hyperactivity and reduced prepulse inhibition in a volume-dependent manner. However, THIP did not reverse the deficits in cued fear or startle response.[58] Current studies show that enhancing GABAergic transmission can correct specific behavioral phenotypes of the *Fmr1*-KO mouse, further supporting focus on the GABAergic system and, specifically, tonic inhibition, which might be important for correcting or ameliorating specific behaviors in FXS.[47,49,58]

Gaboxadol reached a Phase III trial before its cessation because of side effects, such as hallucination and disorientation. Due to these safety concerns and lack of efficacy, work on the drug was discontinued in 2007; however, recent studies in animal models of ASD have shown that gaboxadol is effective in rescuing neurophysiological and behavioral deficits.[58,59] Further studies in fragile X animal models are necessary to provide cumulative evidence in the efficacy and safety of gaboxadol. Currently, there are no studies in individuals with FXS.

Vigabatrin

Vigabatrin is an antiepileptic and analog of GABA (although not an agonist) that inhibits the catabolism of GABA by irreversibly inhibiting GABA transaminase. It has been found that the half-life of biologic activity is far longer than the elimination half-life,[60,61] and there is no range of target concentrations because there was no difference between the serum concentration levels of responders and those of nonresponders.[61] In addition, the duration of action is more a function of the GABA transaminase resynthesis rate.[62] Vigabatrin has been approved by the US Food and Drug Administration (FDA) for use in patients with refractory complex partial seizures, but the rentinal toxicity of the medication limits it's use to those who have not responded to other treatments.[63] However, it is recommended when the benefits outweigh the side effects in individuals with intractable seizures. There are no studies in FXS animal models or clinical trials in individuals with FXS.

Arbaclofen

One compound shown to influence GABA regulation in the *Fmr1*-KO mouse is arbaclofen, a $GABA_B$ agonist developed by Seaside Therapeutics. Arbaclofen is a receptor agonist and active enantiomer of racemic baclofen, which presynaptically blocks glutamate release, thereby decreasing the overactivation of the glutamatergic pathways. In *Fmr1*-KO mice, arbaclofen corrected elevated protein synthesis in the hippocampus; reduced elevated AMPA (α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor internalization to wild-type values; decreased mRNA translation in the cortex; and corrected the increased spine density prevalent in the mouse phenotype.[64]

In pediatric, adolescent, and adult patients with FXS, arbaclofen was administered in a randomized, double-blind, placebo-controlled crossover study. The primary outcome measure, the

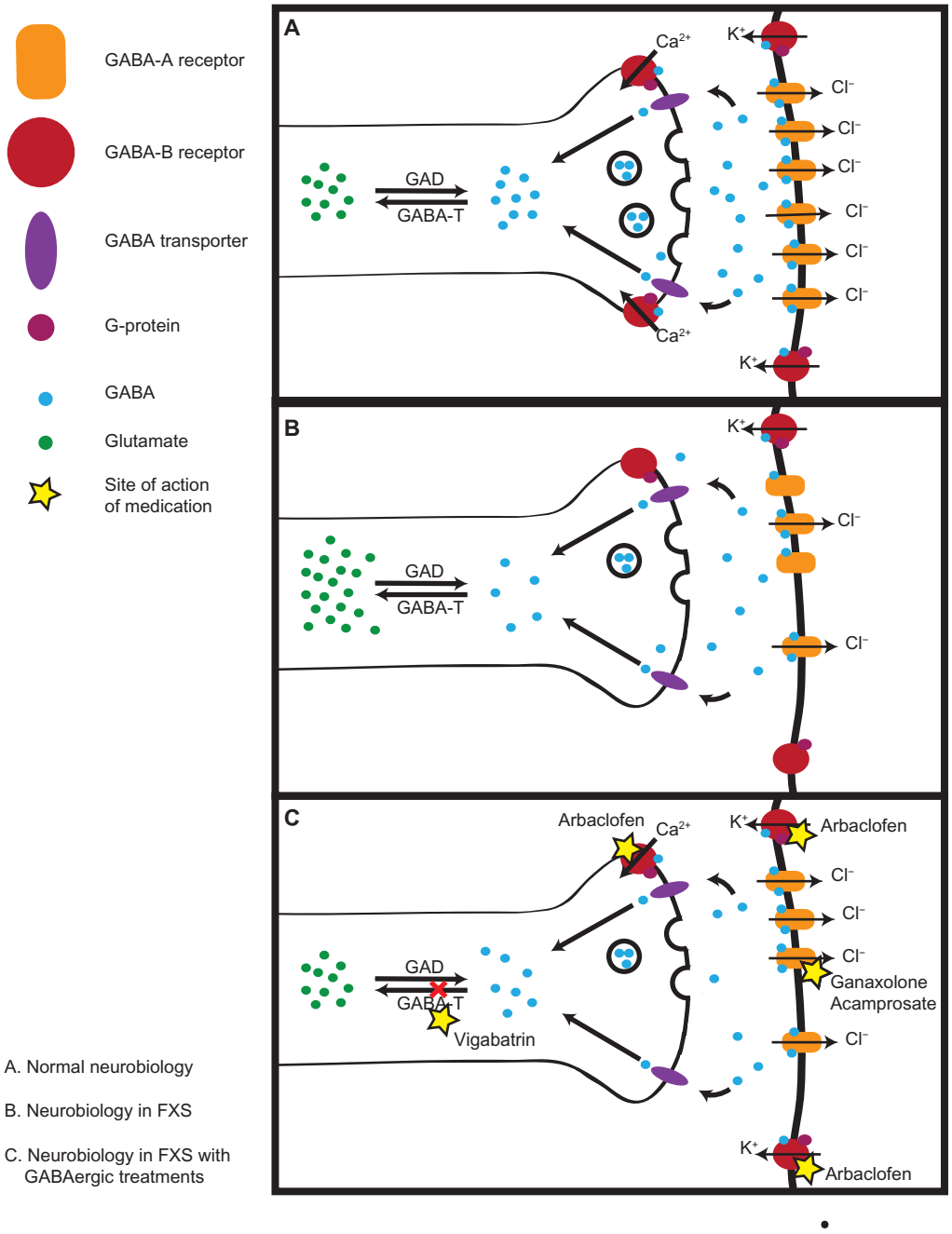

Figure 1 Neurobiology in FXS.

Notes: (**A**) In the normal neurobiology of the synapse, FMRP regulates the expression of GABA receptors and the metabolism and catabolism of GABA. (**B**) The affected neurobiology in FXS, with reduced GABA synthesis and transport, fewer $GABA_A$ and $GABA_B$ receptors, and therefore overall reduced GABAergic activity. (**C**) The neurobiology in FXS with GABAergic treatments. The sites of action for the medications are indicated by stars on the various receptors and enzymes. Arbaclofen focuses on the presynaptic $GABA_B$ receptors to increase their activity with a secondary inhibitory effect on the presynaptic release of glutamate. Ganaxolone, acamprosate, and riluzole all work on the $GABA_A$ receptors to increase the reduced activity. Ganaxolone, however, has higher affinity to the extrasynaptic δ subunit containing $GABA_A$ receptors. Vigabatrin reduces the resynthesis of GABA to glutamate, which is already increased in FXS.

Abbreviations: FMRP, fragile X mental retardation protein; FXS, fragile X syndrome; GABA, gamma-aminobutyric acid; GABA-T, gamma-aminobutyric acid transaminase; GAD, glutamic acid decarboxylase.

Aberrant Behavior Checklist-Community Edition, a 58-item behavioral questionnaire, did not show reduction in any of the subscales compared to placebo[42] and, due to resource limitations, Seaside Therapeutics had to terminate its arbaclofen program.[65] However, a post hoc analysis saw a full study improvement on the Aberrant Behavior Checklist Social Avoidance subscale, which was an algorithm developed specifically to assess behavioral function in patients with FXS.[66] In addition, a subgroup of 27 patients with more severe social impairment treated with arbaclofen showed improvement on all global measures, as well as on

the Vineland Socialization subscale, which is a semi-structured parent interview that assesses adaptive behavior. There were also trends of improvement seen on parent-nominated problem behaviors in the visual analog scale as well as on multiple global measures.[42] This demonstrates the need to use biological subtyping, particularly in a heterogeneous group of patients, such as those with ASD. For the subgroup of patients with FXS who did very well with arbaclofen, it became a problem to obtain the drug once the company folded, and the withdrawal from arbaclofen was very difficult for many families. Overall, arbaclofen was well tolerated, with sedation and headache being the most commonly reported side effects.[42]

Riluzole

Riluzole is a medication approved for the treatment of amyotrophic lateral sclerosis, which blocks voltage-gated Na^+ channels and selectively depresses glutamate over GABA release and which, at higher concentrations, can potently increase postsynaptic $GABA_A$ response in hippocampal neurons.[67] Due to the GABAergic dysregulation and epilepsy shown in patients with FXS, it was proposed that riluzole could act as a beneficial treatment for FXS. An open-label study of riluzole in patients with FXS showed a clinical response in only one of six patients, with significant improvement as measured on the ADHD Rating Scale-IV.[43] Riluzole administration was also associated with significant correction of ERK activation time in all subjects. Overall, the medication was well tolerated and showed non-clinically significant increases in liver function tests,[43] a notable side effect of the medication.

Tiagabine

Tiagabine, a nipecotic acid derivative, is another antiepileptic that reduces neuronal and astrocytic uptake of GABA.[68] Sound-induced convulsions in DBA/2 mice – which, notably, had no sedation or motor debilitation[70] – were corrected when tiagabine was administered;[69] however, there have not been any studies assessing the efficacy of tiagabine in treating FXS. While it may be used to control epilepsy in patients with FXS, other antiepileptic medications seem to have more potent treatment responses.[71]

Conclusion

Numerous GABAergic compounds are potential treatments for FXS; however, considerably more basic and translational research is needed. The current studies are mostly open-label treatment options with small sample sizes of patients in a wide age range. Larger, double-blind, placebo-controlled trials are needed to assess the efficacy of these treatments against the placebo in order to better establish the treatment profile of each medication. The largest double-blind, placebo-controlled study using a GABAergic compound in FXS used arbaclofen. Despite trends in a positive treatment response on secondary outcome measures, Seaside Therapeutics could not continue with additional studies focusing on the secondary outcome measures due to limited resources. This outcome brings to light the difficulty of establishing efficacy of targeted treatments in this population.[42,72]

A number of new treatments are also being studied in clinical trials after promising efficacy studies in the animal models of FXS.[73] These include lovastatin, which lowers ERK phosphorylation in the mTOR pathway, and an insulin-like growth factor 1 analog made by Neuren (Neuren Pharmaceuticals Limited, Camberwell, VIC, Australia). Other treatment trials are in the pipeline but have yet to come to clinical trials.[74]

There are a number of barriers to new treatments, including the time and expense of carrying out the toxicity studies in animals and humans and subsequent multicenter human trials to demonstrate efficacy. Although FXS is a single-gene disorder, there is significant heterogeneity in clinical involvement and response to treatment. For many trials, approximately 30% respond well, but this may not be adequate to demonstrate overall efficacy, which prohibits FDA approval for marketing. The lack of biomarkers that would predict efficacy is greatly needed so that a "likely to respond subgroup" could be identified.

For example, the AFQ056 compound by Novartis appears to be most effective for those who are fully methylated, but additional biomarkers are needed.[75] In addition, outcome measures that are quantitative and relate to central nervous system function or molecular changes and do not depend on questionnaires from the family would be useful to decreasing the placebo effect. Event-related potential paradigms of cognitive processing would also be useful, and we have seen a positive effect using the oddball paradigm and habituation task in children treated with minocycline in a controlled trial.[76]

The placebo effect is high, owing to the overwhelming need for effective interventions, which is further impacted by the difficulty of obtaining objective outcome measures that accurately assess a behavioral treatment response. A majority of the improvement measures were based on parent or caregiver response,[42,66] which is subjective. More objective outcome measures are needed, not only for GABAergic medications, but for all clinical trials that focus on neurodevelopmental indications. Biomarkers relating specifically to GABA upregulation in patients who did improve on the arbaclofen medication would be a vital tool for establishing efficacy against placebo.

Combined clinical trials using two drugs or a drug with an intervention program may show positive outcomes for FDA approval. A combined clinical trial of lovastatin with language intervention for individuals with FXS has been supported by the National Institute of Child Health and Human Development.[77] GABA downregulation and mGluR5 upregulation are thought to be the major problems in FXS, so interventions in both of these pathways will be critical for an overall treatment program. Ganaxolone is currently undergoing a clinical trial in FXS and is considered to have the best potential as a GABA agent because it specifically targets the $GABA_A$ pathway, which is most dysregulated in FXS. If efficacy in FXS is demonstrated and there is FDA approval for an FXS indication, then it will be studied in ASD and related disorders. Research needs to start focusing on multifaceted treatment options that combine multiple pharmacological agents and/or behavioral interventions due to the complex nature of the disease, but this may be difficult prior to FDA approval for each compound. Studies looking at behavioral and pharmacological interventions are planned for other targeted treatment options in FXS, including the mGluR5 antagonists and lovastatin. No studies are currently planned for multiple GABAergic medications or GABAergic medications with behavioral interventions, which would be highly useful and may provide more outcome measures with which to gauge treatment response.

In addition to difficulties in outcome measures, it should also be noted that reversing the behavioral and intellectual abilities in FXS is more difficult in adults because they are not typically in a learning program and because the neurobiological abnormalities may be less reversible over time. More intensive learning programs are required, although the improvements seen in the adult mouse with FXS after treatment with a long-acting mGluR5 antagonist were remarkable[78] and gave hope to many of the families that have adult offspring with FXS. Owing to the process involved in obtaining FDA approval for medications, companies must establish safety and efficacy in the adult population prior to moving into younger age groups; as such, the low efficacy of adult studies prevents the medication from moving to the younger age groups, who are more likely to see more robust treatment responses.

It is important to remember that synaptic connections that are strengthened by either a GABA agonist or an mGluR5 antagonist also require an intensive learning environment to strengthen these connections. Although most school-aged children with FXS are receiving special education support in addition to speech and language therapy and occupational therapy, their programs can usually be enhanced by the use of digital learning programs that can be accessed on a tablet device. There are a variety of software applications that have been developed for individuals with ASD, and these programs are likely to also be helpful for those with FXS.[79]

There are many currently used medications that are helpful in FXS that will likely continue to be helpful even after GABA agonists and mGluR5 antagonists are more widely used clinically. These medications include sertraline, a selective serotonin reuptake inhibitor, that can be started at a young age and has been helpful in improving the language trajectory of toddlers with FXS.[80] Another targeted treatment that can be effective at an early age is minocycline, which is an antibiotic that lowers the level of matrix metalloproteinase 9 which is elevated in FXS.[81,82] High levels of matrix metalloproteinase 9 interfere with the development of synaptic connections, and a controlled trial of minocycline in children between the ages of 3 and 17 years (doses ranging from 25 to 100 mg per day) recently demonstrated efficacy on the CGI-I in improving overall behavior and on the visual analog scale for improving mood and anxiety.[81] Side effects of minocycline include graying of the permanent teeth if given before 8 years of age, and graying or darkening of tissue such as in the gums and nail beds at any age. On rare occasions, minocycline can cause a lupus-like syndrome with a rash or swollen joints or pseudotumor cerebri leading to a severe headache; if these problems occur, minocycline should be discontinued immediately.[81] Once children with FXS reach 5 years of age, they usually have a positive response to a stimulant for their ADHD symptoms; for those who do not respond to a stimulant, then an alpha agonist such as guanfacine can be very helpful.[32] Lastly, melatonin has also been helpful for sleep disturbances in FXS, particularly in young children.[83] Overall, a GABA agonist will add significantly to the treatment regimen in children and adults with FXS, although we do not yet know if improvements will be seen only in behavior or if cognitive deficits will also improve with long-term use.

Acknowledgments

Funding for this chapter includes NICHD grant HD 036071, the Autism Research Training Program (MH073124), Department of Defense grant PR101054, support from the Health and Human Services Administration on Developmental Disabilities (grant 90DD05969), and the National Center for Advancing Translational Research (UL1 TR000002).

Disclosure

Dr Hagerman has received funding from Novartis Pharmaceuticals, F Hoffmann-La Roche, Seaside Therapeutics and Neuren Pharmaceuticals Limited to carry out treatment trials in fragile X syndrome and autism. She has also consulted with F Hoffmann-La Roche/Genentech, and Novartis Pharmaceuticals regarding treatment studies in fragile X syndrome. All authors have participated in clinical trials of ganaxolone and arbaclofen for fragile X syndrome.

References

1. Belmonte MK, Bourgeron T. Fragile X syndrome and autism at the intersection of genetic and neural networks. *Nat Neurosci*. 2006;9(10):1221–1225.
2. Bretherick KL, Fluker MR, Robinson WP. FMR1 repeat sizes in the gray zone and high end of the normal range are associated with premature ovarian failure. *Hum Genet*. 2005;117(4):376–382.
3. Hagerman R, Hagerman P. Advances in clinical and molecular understanding of the FMR1 premutation and fragile X-associated tremor/ataxia syndrome. *Lancet Neurol*. 2013;12(8):786–798.
4. Bourgeois JA, Seritan AL, Casillas EM, et al. Lifetime prevalence of mood and anxiety disorders in fragile X premutation carriers. *J Clin Psychiatry*. 2011;72(2):175–182.
5. Winarni TI, Chonchaiya W, Sumekar TA, et al. Immune-mediated disorders among women carriers of fragile X premutation alleles. *Am J Med Genet A*. 2012;158a(10):2473–2481.
6. Au J, Akins RS, Berkowitz-Sutherland L, et al. Prevalence and risk of migraine headaches in adult fragile X premutation carriers. *Clin Genet*. 2013;84(6):546–551.
7. Hamlin AA, Sukharev D, Campos L, et al. Hypertension in FMR1 premutation males with and without fragile X-associated tremor/ataxia syndrome (FXTAS). *Am J Med Genet A*. 2012;158A(6):1304–1309.
8. Sullivan SD, Welt C, Sherman S. FMR1 and the continuum of primary ovarian insufficiency. *Semin Reprod Med*. 2011;29(04):299–307.
9. Conde V, Palomar FJ, Lama MJ, et al. Abnormal GABA-mediated and cerebellar inhibition in women with the fragile X premutation. *J Neurophysiol*. 2013;109(5):1315–1322.
10. Farzin F, Perry H, Hessl D, et al. Autism spectrum disorders and attention-deficit/hyperactivity disorder in boys with the fragile X premutation. *J Dev Behav Pediatr*. 2006;27(Suppl 2):S137–S144.
11. Leigh MJS, Hagerman RJ, Hessl D. Fragile X Syndrome. In: Hansen RL, Rogers SJ, editors. *Autism and Other Neurodevelopmental Disorders*. Arlington: American Psychiatric Publishing; 2013:57–76.
12. Coffee B, Keith K, Albizua I, et al. Incidence of fragile X syndrome by newborn screening for methylated FMR1 DNA. *Am J Hum Genet*. 2009;85(4):503–514.
13. Crawford DC, Acuña JM, Sherman SL. FMR1 and the fragile X syndrome: human genome epidemiology review. *Genet Med*. 2001;3(5):359–371.
14. Sorensen PL, Gane LW, Yarborough M, Hagerman RJ, Tassone F. Newborn screening and cascade testing for FMR1 mutations. *Am J Med Genet A*. 2013;161(1):59–69.
15. Allen EG, He W, Yadav-Shah M, Sherman SL. A study of the distributional characteristics of FMR1 transcript levels in 238 individuals. *Hum Genet*. 2004;114(5):439–447.
16. Ludwig AL, Espinal GM, Pretto DI. CNS expression of murine fragile X protein (FMRP) as a function of CGG-repeat size. *Hum Mol Genet*. Epub February 11, 2014.
17. Bhakar AL, Dölen G, Bear MF. The pathophysiology of fragile X (and what it teaches us about synapses). *Annu Rev Neurosci*. 2012;35: 417–443.
18. Brown V, Jin P, Ceman S, et al. Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell*. 2001;107(4):477–487.
19. Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. *Trends Neurosci*. 2004;27(7):370–377.
20. Moy SS, Nadler JJ. Advances in behavioral genetics: mouse models of autism. *Mol Psychiatry*. 2008;13(1):4–26.
21. Rubenstein J, Merzenich M. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav*. 2003;2(5):255–267.
22. Bagni C, Tassone F, Neri G, Hagerman R. Fragile X syndrome: causes, diagnosis, mechanisms, and therapeutics. *J Clin Invest*. 2012;122(12):4314–4322.
23. Fatemi S, Folsom T, Rooney R, Thuras P. mRNA and protein expression for novel $GABA_A$ receptors θ and ρ2 are altered in schizophrenia and mood disorders; relevance to FMRP-mGluR5 signaling pathway. *Transl Psychiatry*. 2013;3(6):e271.
24. Dölen G, Carpenter RL, Ocain TD, Bear MF. Mechanism-based approaches to treating fragile X. *Pharmacol Ther*. 2010;127(1): 78–93.
25. Berry-Kravis E, Knox A, Hervey C. Targeted treatments for fragile X syndrome. *J Neurodev Disord*. 2011;3(3):193–210.
26. Padgett CL, Slesinger PA. GABAB receptor coupling to G-proteins and ion channels. *Adv Pharmacol*. 2010;58:123–147.
27. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci*. 2005;6(3):215–229.
28. Cardin JA, Carlén M, Meletis K, et al. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature*. 2009;459(7247):663–667.
29. Wang LW, Berry-Kravis E, Hagerman RJ. Fragile X: leading the way for targeted treatments in autism. *Neurotherapeutics*. 2010;7(3):264–274.
30. Sohal VS, Zhang F, Yizhar O, Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature*. 2009;459(7247):698–702.
31. Berry-Kravis E, Grossman AW, Crnic LS, Greenough WT. Understanding fragile X syndrome. *Current Paediatrics*. 2002;12(4):316–324.
32. Hagerman RJ, Berry-Kravis E, Kaufmann WE, et al. Advances in the treatment of fragile X syndrome. *Pediatrics*. 2009;123(1):378–390.
33. Adusei DC, Pacey LK, Chen D, Hampson DR. Early developmental alterations in GABAergic protein expression in fragile X knockout mice. *Neuropharmacology*. 2010;59(3):167–171.
34. Liao CW, Lien C. Estimating intracellular Ca2+ concentrations and buffering in a dendritic inhibitory hippocampal interneuron. *Neuroscience*. 2009;164(4):1701–1711.
35. Olmos-Serrano JL, Paluszkiewicz SM, Martin BS, Kaufmann WE, Corbin JG, Huntsman MM. Defective GABAergic neurotransmission and pharmacological rescue of neuronal hyperexcitability in the amygdala in a mouse model of fragile X syndrome. *J Neurosci*. 2010;30(29):9929–9938.
36. Curia G, Papouin T, Séguéla P, Avoli M. Downregulation of tonic GABAergic inhibition in a mouse model of fragile X syndrome. *Cereb Cortex*. 2009;19(7):1515–1520.
37. D'Hulst C, Heulens I, Brouwer JR, et al. Expression of the GABAergic system in animal models for fragile X syndrome and fragile X associated tremor/ataxia syndrome (FXTAS). *Brain Res*. 2009;1253:176–183.
38. Gantois I, Vandesompele J, Speleman F, et al. Expression profiling suggests underexpression of the $GABA_A$ receptor subunit delta in the fragile X knockout mouse model. *Neurobiol Dis*. 2006;21(2):346–357.
39. Pacey LKK, Heximer SP, Hampson DR. Increased GABA(B) receptor-mediated signaling reduces the susceptibility of fragile x knockout mice to audiogenic seizures. *Mol Pharmacol*. 2009;76(1):18–24.
40. Kim SY, Burris J, Bassal F, et al. Fear-specific amygdala function in children and adolescents on the fragile x spectrum: a dosage response of the FMR1 gene. *Cereb. Cortex*. 2014;24(3):600–613.
41. Gibson JR, Bartley AF, Hays SA, Huber KM. Imbalance of neocortical excitation and inhibition and altered UP states reflect network hyperexcitability in the mouse model of fragile X syndrome. *J Neurophysiol*. 2008;100(5):2615–2626.

42. Berry-Kravis EM, Hessl D, Rathmell B, et al. Effects of STX209 (arbaclofen) on neurobehavioral function in children and adults with fragile X syndrome: a randomized, controlled, phase 2 trial. *Sci Transl Med*. 2012;4(152):152ra127.
43. Erickson CA, Weng N, Weiler IJ, et al. Open-label riluzole in fragile X syndrome. *Brain Res*. 2011;1380:264–270.
44. Erickson CA, Wink LA, Ray B, et al. Impact of acamprosate on behavior and brain-derived neurotrophic factor: an open-label study in youth with fragile X syndrome. *Psychopharmacology (Berl)*. 2013; 228(1):75–84.
45. Carter RB, Wood PL, Wieland S, et al. Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3β-hydroxy-3β-methyl-5α-pregnan-20-one), a selective, high-affinity, steroid modulator of the γ-aminobutyric acidA receptor. *J Pharmacol Exp Ther*. 1997;280(3):1284–1295.
46. Kerrigan JF, Shields WD, Nelson TY, et al. Ganaxolone for treating intractable infantile spasms: a multicenter, open-label, add-on trial. *Epilepsy Res*. 2000;42(2):133–139.
47. Heulens I, D'Hulst C, Van Dam D, De Deyn PP, Kooy RF. Pharmacological treatment of fragile X syndrome with GABAergic drugs in a knockout mouse model. *Behav Brain Res*. 2012;229(1):244–249.
48. D'Hulst C, De Geest N, Reeve SP, et al. Decreased expression of the $GABA_A$ receptor in fragile X syndrome. *Brain Res*. 2006;1121(1): 238–245.
49. Chang S, Bray SM, Li Z, et al. Identification of small molecules rescuing fragile X syndrome phenotypes in Drosophila. *Nat Chem Biol*. 2008;4(4):256–263.
50. Pieribone VA, Tsai J, Soufflet C, et al. Clinical evaluation of ganaxolone in pediatric and adolescent patients with refractory epilepsy. *Epilepsia*. 2007;48(10):1870–1874.
51. Marinus Pharmaceuticals. Ganaxolone Treatment in Children With Fragile X Syndrome. Available from: http://clinicaltrials.gov/ct2/show/NCT01725152. NLM identifier: NCT01725152. Accessed March 24, 2014.
52. Nusser Z, Mody I. Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J Neurophysiol*. 2002;87(5): 2624–2628.
53. Cope DW, Hughes SW, Crunelli V. GABAA receptor-mediated tonic inhibition in thalamic neurons. *J Neurosci*. 2005;25(50):11553–11563.
54. Brown N, Kerby J, Bonnert T, Whiting P, Wafford K. Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3) delta GABA(A) receptors. *Br J Pharmacol*. 2002;136(7):965–974.
55. Wohlfarth KM, Bianchi MT, Macdonald RL. Enhanced neurosteroid potentiation of ternary GABA(A) receptors containing the delta subunit. *J Neurosci*. 2002;22(5):1541–1549.
56. Haas KF, Macdonald RL. GABAA receptor subunit gamma2 and delta subtypes confer unique kinetic properties on recombinant GABAA receptor currents in mouse fibroblasts. *J Physiol*. 1999;514(Pt 1):27–45.
57. Deng PY, Rotman Z, Blundon JA, et al. FMRP regulates neurotransmitter release and synaptic information transmission by modulating action potential duration via BK channels. *Neuron*. 2013;77(4):696–711.
58. Olmos-Serrano JL, Corbin JG, Burns MP. The GABA(A) receptor agonist THIP ameliorates specific behavioral deficits in the mouse model of fragile X syndrome. *Dev Neurosci*. 2011;33(5):395–403.
59. Egawa K, Kitagawa K, Inoue K, et al. Decreased tonic inhibition in cerebellar granule cells causes motor dysfunction in a mouse model of angelman syndrome. *Sci Transl Med*. 2012;4(163):163ra157.
60. Browne TR. Pharmacokinetics of antiepileptic drugs. *Neurology*. 1998;51(5 Suppl 4):S2–S7.
61. Browne T, Szabo G. New pharmacokinetic methods for the study of antiepileptic medications of the 1990s. *Epilepsia*. 1990;32:S66–S73.
62. Lindberger M, Luhr O, Johannessen SI, Larsson S, Tomson T. Serum concentrations and effects of gabapentin and vigabatrin: observations from a dose titration study. *Ther Drug Monit*. 2003;25(4):457–462.
63. Podboraczynska-Jodkoi K, Lubinski W, Hampel-Osipowicz E. [Retinal dysfunction in patients treated with vigabatrin]. *Klin. Oczna*. 2007;109(1–3):85–88. Polish.
64. Henderson C, Wijetunge L, Kinoshita MN, et al. Reversal of disease-related pathologies in the fragile X mouse model by selective activation of GABAB receptors with arbaclofen. *Sci Transl Med*. 2012; 4(152):152ra128.
65. Thomson Reuters. Key trial of Seaside autism drug fails to show benefit [press release]. New York, NY: Thomson Reuters; 2013 [May 1]. Available from: http://www.reuters.com/article/2013/05/01/us-autism-drug-idUSBRE9400NT20130501. Accessed May 05, 2014
66. Sansone SM, Widaman KF, Hall SS, et al. Psychometric study of the Aberrant Behavior Checklist in Fragile X Syndrome and implications for targeted treatment. *J Autism Dev Disord*. 2012;42(7):1377–1392.
67. He Y, Benz A, Fu T, et al. Neuroprotective agent riluzole potentiates postsynaptic GABA(A) receptor function. *Neuropharmacology*. 2002;42(2):199–209.
68. Leach JP, Brodie MJ. New antiepileptic drugs – an explosion of activity. *Seizure*. 1995;4(1):5–17.
69. Suzdak PD, Jansen JA. A review of the preclinical pharmacology of tiagabine: a potent and selective anticonvulsant GABA uptake inhibitor. *Epilepsia*. 1995;36(6):612–626.
70. Nielsen EB, Suzdak PD, Andersen KE, Knutsen LJ, Sonnewald U, Braestrup C. Characterization of tiagabine (NO-328), a new potent and selective GABA uptake inhibitor. *Eur J Pharmacol*. 1991; 196(3):257–266.
71. Berry–Kravis E. Epilepsy in fragile X syndrome. *Dev Med Child Neurol*. 2002;44(11):724–728.
72. National Fragile X Foundation. Seaside Therapeutics Announces End of Arbaclofen (STX209) Extension Study [press release]. Walnut Creek, CA: National Fragile X Foundation; 2013 [May 16]. Available from: http://www.fragilex.org/2013/research/news-reports-and-commentaries/seaside-therapeutics-announces-end-of-arbaclofen-stx209-extension-study/. Accessed May 06, 2014.
73. Berry-Kravis E. Mechanism-based treatments in neurodevelopmental disorders: fragile X syndrome. *Pediatr Neurol*. 2014;50(4):297–302.
74. Braat S, Kooy RF. Fragile X syndrome neurobiology translates into rational therapy. *Drug Discovery Today*. 2014;19(4):510–519.
75. Jacquemont S, Curie A, des Portes V, et al. Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. *Sci Transl Med*. 2011;3(64):64ra1.
76. Schneider A, Leigh MJ, Adams P, et al. Electrocortical changes associated with minocycline treatment in fragile X syndrome. *J Psychopharmacol*. 2013;27(10):956–963.
77. University of California, Los Angeles. Trial to evaluate the safety of Lovastatin in individuals with neurofibromatosis Type I (NF1). Available from: http://clinicaltrials.gov/show/NCT00352599. NLM identifier: NCT00352599. Accessed May 26, 2014.
78. Michalon A, Sidorov M, Ballard TM, et al. Chronic pharmacological mGlu5 inhibition corrects fragile X in adult mice. *Neuron*. 2012;74(1): 49–56.
79. Chen W. Multitouch tabletop technology for people with autism spectrum disorder: a review of the literature. *Procedia Comput Sci*. 2012;14:198–207.
80. Winarni TI, Schneider A, Borodyanskara M, Hagerman RJ. Early intervention combined with targeted treatment promotes cognitive and behavioral improvements in young children with fragile x syndrome. *Case Rep Genet*. 2012;2012:280813.
81. Leigh MJ, Nguyen DV, Mu Y, et al. A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile x syndrome. *J Dev Behav Pediatr*. 2013;34(3):147–155.
82. Dziembowska M, Pretto DI, Janusz A, et al. High MMP-9 activity levels in fragile X syndrome are lowered by minocycline. *Am J Med Genet A*. 2013;161A(8):1897–1903.
83. Wirojanan J, Jacquemont S, Diaz R, et al. The efficacy of melatonin for sleep problems in children with autism, fragile X syndrome, or autism and fragile X syndrome. *J Clin Sleep Med*. 2009;5(2):145–150.

EXPERT OPINION

Targeted treatments in fragile X syndrome

Emma B Hare, Randi J Hagerman & Reymundo Lozano†
UC Davis MIND Institute, Sacramento, CA, USA

Introduction: Mouse models of fragile X syndrome (FXS), the most common cause of inherited intellectual disability, show decreased GABAergic input and overactive metabotropic glutamate receptor (mGluR) system, which result in an imbalance between excitatory (Glutamate) and inhibitory (GABA) systems in the brain. The cognitive, behavioral and neurological impairments require the development of targeted treatments that focus on regulating the mGluR and GABA pathways. The increasing literature on this topic and controversial results suggest the need for a comprehensive review of the literature and our expert opinion.

Areas covered: This article includes an extended review of the literature and comprehensive analysis of the current studies in humans and animal models with FXS.

Expert opinion: Our expert opinion is that these orphan drugs will help reduce, but not completely ameliorate, the behavioral phenotype of FXS through improved synaptic plasticity. Single-compound treatment may not be enough for the most affected individuals with FXS and although the orphan drugs may improve more generalized symptoms of FXS compared to symptom-based treatments, the most effective treatment response will require a multifaceted approach starting early in life using multiple pharmacological and behavioral interventions. More research is needed to assess how combinations of these medications, along with behavioral intervention and educational opportunities, will best reverse phenotypic features of FXS. The clinical trials of FXS are challenging, but offers hope for effective treatments for this syndrome and other related neurodevelopmental disorders, including autism.

Keywords: AFQ056, arbaclofen, fragile X mental retardation 1, fragile X syndrome, ganaxolone, lovastatin, mavoglurant, minocycline, RO4917523, targeted treatments

Expert Opinion on Orphan Drugs [Early Online]

1. Introduction

Fragile X syndrome (FXS) and associated disorders are well characterized, and the understanding of the genetic mutation and molecular abnormalities have been defined, leading to the development of numerous targeted treatments focusing on the metabotropic glutamate receptor 5 (mGluR5) and GABA pathways. In addition to the development of new compounds is the reconsideration of FDA-approved medications for the treatment of FXS. There are a number of medications currently available for treating symptoms of FXS, such as stimulants for attention deficit hyperactivity disorder (ADHD), selective serotonin reuptake inhibitors for treating anxiety in addition to atypicals (aripiprazole and risperidone) for treating aggression. In addition, there are several targeted treatments focused on reversing neurobiological changes in FXS that hold the potential for treating core features of FXS. There is an adequate size of literature on animal studies and small-scale, open-label trials, assessing the efficacy of these medications; however, large,

informa
healthcare

10.1517/21678707.2014.903795 © 2014 Informa UK, Ltd. e-ISSN 2167-8707

Article highlights.

- The neurobiological studies in animal models of FXs show an imbalance between excitatory (Glutamate) and inhibitory (GABA) systems in the brain.
- FMRP is a major regulator of RNA translation in the brain.
- Several compounds that regulat the GABA and mGlur system have been studied in animal models of FXS and individuals with FXS.
- The trials for FXS are challenging, but provide of for the treatment of this syndrome.
- The treatments of FXS are leading the way to treat other neurodevelopmental disorders including autism.
- Biomarkers may be excellent candidates in indentifying responders for specific treatments and suceptibility to side effects.
- Further studies are necesary to identify better scaoutcome measurements to identify the eficacy of targeted treatments.

This box summarizes key points contained in the article.

double-blind, placebo-controlled trials are limited, and robust outcome measures are lacking. This paper reviews the currently available literature on the two main targeted treatments developed for FXS (mGluR5 antagonists and GABA agonists), reviews the FDA-approved medications that have the potential to impact FX-associated pathways and looks to future directions for pharmacological treatment of FXS. First, this article will provide a short review of FXS, associated disorders and the neurobiology of the disease followed by an explanation of the animal models, which have been instrumental in both characterizing the disease and developing potential interventions. This will be followed by summaries of the published literature on the orphan drugs and other potential pharmaceuticals, concluding with an expert opinion on the status of targeted treatments in FXS.

The fragile X mental retardation 1 gene (*FMR1*) located on the long arm of the X chromosome at Xq27.3 is responsible for both FXS and fragile X-associated disorders (FADs). Typical individuals have a trinucleotide expansion of 5 – 44 CGG repeats in the 5′ untranslated region near the promoter, premutation carriers have 55 – 200 CGG repeats and individuals with the full mutation leading to FXS have > 200 CGG repeats and some degree of methylation. The full mutation leads to a deficit of the FMR1 protein (FMRP) which causes FXS, the most common form of inherited intellectual disability (ID), the most common genetic (single gene) cause of autism. FXS affects approximately 1 in 2500 to 1 in 5000 individuals [1,2]. The premutation is estimated to be present in 1 in 130 – 250 females and 1 in 250 – 810 males [3].

The fully expanded trinucleotide sequence with methylation silences the transcription, thus leading to the lack of the production of FMRP, which is critical for synaptic function and development. The low or lack of FMRP leads to a characteristic array of physical, behavioral, cognitive and neurological symptoms, which makeup the phenotype of FXS. However, the manifestation of the behavioral, cognitive and physical features range from mild to severe and many individuals with FXS do not present with the typical dysmorphic features of prominent ears and a long face. In consideration of the variability of the phenotype, the American College of Medical Genetics recommends genetic testing for all boys with ID and/or autism and females with a characteristic family history of FXS and/or FADS. Typically, this is because females are not as affected due to their second X chromosome, which accounts for closer to normal FMRP production compared to males with FXS. Individuals with FXS often have prominent ears, long face, soft skin, hyperextensible finger joints and macroorchidism with some characteristics not developing until adolescence. Behavioral problems include anxiety, social avoidance, poor eye contact, ADHD, perseverative speech and behavior, motor stereotypies, aggression and self-injurious behavior [4]. Cognitive and neurological impairments include developmental delay, executive function deficits, impaired reasoning and visual spatial processing, hypersensitivity to environmental stimuli, disrupted sleep patterns and epilepsy [5-8].

Mutations in the premutation range can lead to FADs with variability in penetrance. Premutation disorders include psychiatric, neurologic, immunologic and endocrine impairments in some individuals, including the well-characterized neurodegenerative disorder, fragile X-associated tremor ataxia syndrome (FXTAS) [9,10], fragile X-associated primary ovarian insufficiency (fragile X-associated premature ovarian insufficiency [FXPOI]-menopause before age 40) [11], depression and anxiety [12,13]. In carriers, the excessive transcriptional activity of the *FMR1* region causes overproduction of mRNA from 2 to 8 times greater than normal, leading to toxic effects in the neuron [14]. In addition, some premutation carriers can have lowered levels of FMRP leading to more significant neurodevelopmental disorders, including ID, autism spectrum disorder (ASD) and sometimes seizures. Remarkably, the neurological and neurodevelopmental disorders associated with the premutation have incomplete penetrance and variable expression so that many carriers do not experience these problems.

FXTAS is a progressive neurodegenerative disorder characterized by intention tremor, gait ataxia, white matter disease and global brain atrophy and has an incidence of about 40% in older male carriers and about 16% of older female carriers [10]. FXPOI is attributed as the most common cause of early menopause and affects approximately 20% of carriers [11]. Depression, anxiety or both are present in roughly 40% of carriers, which may be exacerbated by having a child affected by FXS; however, the presence of these psychiatric problems often occurs prior to having an affected child [13,15-17]. Although treatment options for carriers are also under consideration and development, targeted treatments have been produced primarily for treatment of FXS and are leading the way for targeted treatments in ASD, autism and other neurodevelopmental disorders.

2. Neurobiology

Over the past two decades, the molecular research has led to substantial advances in understanding the neurobiology of FXS and related disorders. FMRP is lowered or absent in FXS and is a selective RNA-binding protein, which regulates the translation of hundreds of mRNAs usually through inhibition [18] and is highly expressed in the brain (neurons) and testis. It is estimated that FMRP binds about 4% of all neurons RNAs and interacts with many other proteins in the brain [19-21]. Therefore, FMRP is a major regulator of translation and not surprisingly plays a role in multiple neurodevelopmental and neuropsychiatric disorders [22].

FMRP contains three main RNA-binding domains; two hnRNP K-homology (KH) domains and one RGG box. In addition, a stem loop SoSLIP motif and U-rich sequences have been proposed to be RNA-binding sites. The I304N point mutation, which is located within the second KH domain, causes severe FXS and suggests that this domain plays an essential role in the FMRP function. FMRP mainly regulates RNA transportation, stabilization and translation. *In vitro* FMRP is part of messenger ribonucleoparticles and regulates dendritic transport of associated mRNAs, which result in the production of protein synthesis at the synapse [18]. In addition, FMRP interacts with several cytoplasmic and nuclear proteins and has been found in granules containing translationally silent preinitiation complexes.

Protein synthesis in the soma, axons, dendrites and post-synaptic sites of the brain is required for long-term depression (LTD), a form of hippocampal synaptic plasticity, which consolidates synaptic activity. Local neuronal protein synthesis promotes synaptic plasticity activation, as well as the activation of different synaptic plasticity states, and it is coordinated by the action of mGluRs [23]. In the *Fmr1* knockout (KO) mice, LTD is significantly increased [18]. This effect on LTD is likely due to dysregulated local protein synthesis and has established the basis of the 'mGluR theory' [24]. The mGluR theory of FXS suggests that the psychiatric and neurologic aspects of the syndrome are due to exaggerated downstream consequences of mGluR5 upregulation and was validated by genetic mouse studies where rescue of several symptoms occurred when the mGluR heterozygous mouse was crossed with the *Fmr1* KO mouse [25]. The *Fmr1* KO also shows excess protein translation in the hippocampus and an excessive number of synaptic proteins [26]. Additionally, FMRP binds and represses the catalytic subunit of phosphoinositide 3-kinase, a signaling molecule downstream of the activation of mGluR5 [27]. The p70 ribosomal S6 kinase 1 (S6K1) is involved in the translation initiation and elongation of proteins and is also a signaling molecule downstream of mGluR5 activation as well as a common modulator of both the mammalian target of rapamycin (mTOR) C1 and extracellular signal-regulated kinases (ERK) signaling. *Fmr1* KO mice were crossed with mice globally lacking S6K1, S6K1 KO, and the resultant *Fmr1*/S6K1KO (dKO) mice showed decreased protein synthesis, decreased mGlur5-dependant LTD, decreased weight gain and lower testicular volume. Of note, testicular volume was not rescued in the *FMR1*/mGlur5 KO mice. The deletion of the *S6K1* gene in the dKO also prevented immature dendritic spine morphology and multiple behavioral phenotypes, including social interaction deficits, impaired novel object recognition and behavioral inflexibility that is typical of FXS. The dKO mice were particularly proficient at reversal learning (the strongest deficit in the *FMR1* KO mouse), but they continue to have hyperactive traits [28].

The main inhibitory system in the brain is GABA. There are two classes of GABA receptors: $GABA_A$ and $GABA_B$. $GABA_A$ receptors are ligand-gated ion channels, whereas $GABA_B$ receptors are G-protein-coupled receptors. $GABA_A$ receptors allow the flow of chloride ions across the membrane, which hyperpolarizes the neuron's membrane and minimizes the effect of any coincident synaptic input. $GABA_B$ receptors hyperpolarized the neuron's membrane by activating G-protein-coupled inwardly rectifying potassium channels [29]. The GABA receptors are very diverse in their subunit composition and their effects are fast and slow persistent-tonic inhibition depending on the grade of stimulation [30].

The GABA system is required for a balanced neuronal activation and network oscillations and for facilitating the movement of information in brain areas involved in cognition [31-33]. An imbalance between the excitatory glutamatergic and the inhibitory GABAergic neurotransmission is proposed to cause the cognitive impairments, anxiety, hyperarousal, autism and epilepsy [34-37]. In fact, the excessive amygdala activation is implicated in the social avoidance and anxiety evident in FXS [38]. Recent studies aim at identifying the defects in the inhibitory GABA system in the *Fmr1* KO mouse. FMRP targets the mRNAs encoding eight different $GABA_A$ receptor subunits ($\alpha1$, $\alpha3$, $\alpha4$, $\beta1$, $\beta2$, $\gamma1$, $\gamma2$ and δ), which were significantly reduced in the cortex of *Fmr1* KO mice, particularly the δ subunit, which represents extrasynaptic (perisynaptic) $GABA_A$ receptors [39]. The *Fmr1* KO mouse exhibits reduced inhibitory postsynaptic currents in the amygdala [40] and subicular neurons [41]. Proteins required for GABA transport and catabolism (GABA-T, SSADH) are also reduced [42,43]. In addition, FMRP in the presynaptic side regulates the expression of GAD, which is the rate-limiting GABA synthesis enzyme [40,42,44]. Therefore, FMRP regulates the expression of the GABA receptors, metabolism and catabolism of GABA, and in its absence there is decreased GABAergic input.

3. Targeted treatments

Discovery of the mGluR5 and GABA impairment in FXS has led to an array of targeted treatments focused on regulating these neural pathways that are disrupted because of FMRP deficiency. While this article will discuss multiple targeted treatments, two in particular have been developed specifically

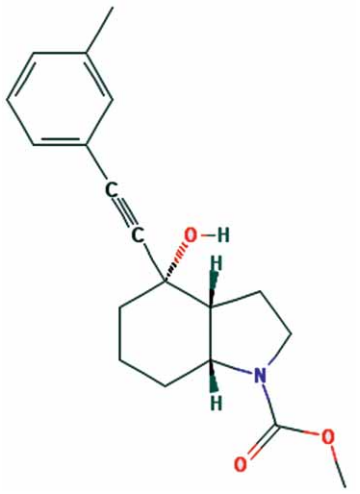

Figure 1. Illustration of mavoglurant; AFQ056.
Adapted from [93].

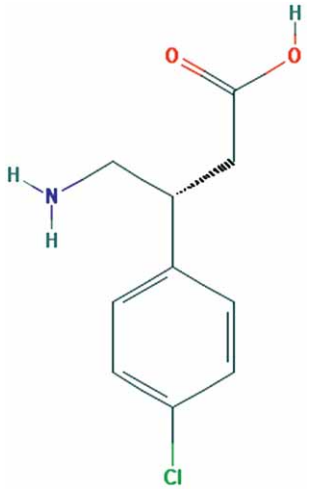

Figure 2. Illustration of arbaclofen.
Adapted from [94].

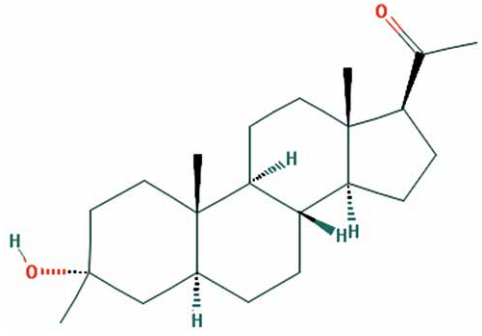

Figure 3. Illustration of ganaxolone.
Adapted from [95].

for FXS and as such are designated as orphan drugs. The discussion of targeted treatments would not be complete without the addition of other medications, which work on similarly impaired pathways. The two orphan medications include mGluR5 antagonists (**Figure 1**) and GABA agonists (**Figures 2** and **3**).

Currently, there are animal models of FXS that include the *Fmr1*-KO mouse and the Drosophila melanogaster *Fmr1* (*dfmr1*). Both animals display phenotypic abnormalities, which are partially or seemingly fully reversed with targeted treatments. The *Fmr1* KO mouse displays enlarged testes, increased locomotor activity, reduced habituation to an open field, increased susceptibility to audiogenic seizures, altered synaptic plasticity, dendritic spine abnormalities, hyperplasticity, behavioral inflexibility in reverse learning and deficits in memory retention and acquisition [45-48]. There are a few different FXS mouse models, which display certain phenotypes in ranging magnitudes, but overall most present with perseverative behavior, audiogenic seizures, reduced anxiety and startle reflexes relative to wild-type controls [49]. Due to these phenotypic similarities with humans, the mouse models function well for gauging treatment response. The Drosophila mutant model has an *Fmr1* gene called *dfrm1*, which is 35% identical and 60% similar to the human *Fmr1* gene and establishes not only a model to study the biological dysregulation in FXS but also a viable organism on which an array of targeted treatments could be tested. The Drosophila mutant model presents with altered circadian rhythm, abnormal synaptic morphology, naïve courtship behavior [50], cognitive deficits in social and memory paradigms and excessive grooming behavior [51]. In summary, both animals have provided sound models for testing the safety and efficacy of new targeted treatments for humans [46].

4. mGluR5 antagonists

4.1 Animal studies

Despite their orphan status, mGluR5 antagonists already include an assortment of compounds under study owing to the importance of this pathway. Numerous mouse studies demonstrated phenotypic rescue of features seen in the *Fmr1* KO mouse and are summarized in **Table 1**. 2-methyl-6-phenylethynyl pyridine hydrochloride (MPEP), 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1*H*-imidazol-4-yl)ethynyl)pyridine (CTEP), fenobam (Neuropharm Ltd), AFQ056 (Mavoglurant; Novartis Pharmaceuticals), RO4917523 (RG7090; Hoffman-La Roche) and STX107 (Seaside Therapeutics) are the main compounds under study; however, Neuropharm and Seaside Therapeutics have closed, thereby discontinuing their studies of FXS, decreasing the pool of possible treatments.

Numerous compounds have been tested in *Fmr1*-KO mouse and *dfmr1* FXS animal models. Drosophila studies have been summarized by McBride *et al.* [51], and they demonstrate phenotypic rescue of neuronal anatomy, behavior

Table 1. Targeted treatment trials for fragile X syndrome.

Compound	Fly phenotypes reversed	Mouse phenotypes reversed	Human studies-completed, active, and planned
MPEP (mGluR5 antagonist)	Mushroom body formation; abnormal male courtship behavior; memory defects [54]	Sensitivity to audiogenic seizures; open-field hyperactivity [55]; PPI defect; neuronal protrusion morphology [56]; prolonged epileptiform synchronized discharges [57]; and low inhibitory serine-phosphorylation of brain GSK3 [58]	
Fenobam (mGluR5 antagonist)		PPI defect; neuronal protrusion morphology [56]	PPI improved and anxiety reduced [65], but company went bankrupt
CTEP (mGluR5 antagonist)		Elevated hippocampal long-term depression; protein synthesis; audiogenic seizures; cognitive deficits; auditory hypersensitivity; aberrant dendritic spine density; overactive ERK and mTOR signaling; and partially corrects macroorchidism [64]	
AFQ056 (mavoglurant; mGluR5 antagonist)			ABC-C and CGI improvement for fully methylated patients [66]. Phase IIB completed for adults and adolescents, and results are pending; future studies planned for pediatric age group and literacy intervention with double-blind AFQ056 treatment
RO4917523 (RG7090; mGluR5 antagonist)			Active Phase II study in adults completed; active Phase II study in adolescents and pediatric patients
STX107 (mGluR5 antagonist)			Completed Phase I safety testing, but company dissolved
Ganaxolone ($GABA_A$ agonist)		Sensitivity to audiogenic seizures [72]	
STX 209 (Arbaclofen; $GABA_B$ agonist)		Excessive mRNA translation in the cortex; increased dendritic spine density; and elevated AMPA receptor internalization [69]	VAS, Vineland-II socialization improvement, and ABC-social avoidance improvement [70]
Lovastatin (HMG-CoA reductase inhibitor)		Excessive hippocampal protein synthesis and epileptogenesis [87]	Double-blind study planned for 2015
Minocycline (tetracycline antibiotic)	Neuroanatomical defects [52]	Abnormal dendritic spine morphology; increased anxiety in the elevated plus maze; decreased exploratory behavior in the Y maze [75]; overexpression of MMP9 [90]; and vocalization deficits [77]	Double-blind crossover: ERP showed temporal N1 and P2 amplitudes reduced; electrocortical habituation improved for auditory stimuli [91]; greater global improvements [80]; open-label behavioral and language improvements [78]; open-label ABC-C irritability, VAS and CGI improvement [79]

ABC-C: Aberrant Behavior Checklist-Community edition; BDNF: Brain-derived neurotrophic factor; CGI: Clinical global improvement scale; CTEP: 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1*H*-imidazol-4-yl)ethynyl)pyridine; ERK: Extracellular signal-regulated kinases; ERP: Event-related potential; mGluR: Metabotropic glutamate receptor; MPEP: Methyl-6-phenylethynyl pyridine hydrochloride; mTOR: Mammalian target of rapamycin; PPI: Prepulse inhibition; VAS: Visual analog scale.

Table 1. Targeted treatment trials for fragile X syndrome (continued).

Compound	Fly phenotypes reversed	Mouse phenotypes reversed	Human studies-completed, active, and planned
CX516 (AMPA receptor positive allosteric modulator)			CX516 Double-blind: no significant improvements [98]
Lithium		Decreased inhibitory serine-phosphorylation of GSK3; decreased hippocampal BDNF; open-field hyperactivity; elevated plus-maze; passive avoidance [58]; hyperactivity; impaired social interactions; deficits on a learning test; anxiety; abnormal dendritic spine morphology [82]	Open-label: ABC-C irritability trend toward improvement; significant improvement on total ABC-C, CGI, VAS and RBANS [84]
Acamprosate			Open-label: 9 of 12 patients showed improved social behavior and reduced inattention/ hyperactivity [74]

ABC-C: Aberrant Behavior Checklist-Community edition; BDNF: Brain-derived neurotrophic factor; CGI: Clinical global improvement scale; CTEP: 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1*H*-imidazol-4-yl)ethynyl)pyridine; ERK: Extracellular signal-regulated kinases; ERP: Event-related potential; mGluR: Metabotropic glutamate receptor; MPEP: Methyl-6-phenylethynyl pyridine hydrochloride; mTOR: Mammalian target of rapamycin; PPI: Prepulse inhibition; VAS: Visual analog scale.

and cognition [51]. MPEP, the first potent negative allosteric modulator of mGluR, reversed mushroom body crossover, peripheral synaptic defects seen as overelaboration of the neuromuscular junction in larvae, neurotransmitter-containing vesicle defects, such as elevated presynaptic vesicle pools, socially impaired naïve courtship, learning decline and both short- and long-term memory deficits [51-54].

MPEP administration in mouse models has further shown significant rescue in a range of phenotypic markers of FXS: correction of sensitivity to audiogenic seizures, open field hyperactivity [55], increased prepulse inhibition (PPI), neuronal protrusion morphology [56], prolonged epileptiform synchronized discharges [57] and low inhibitory serine-phosphorylation of brain GSK3 [58]. *In vivo* MPEP increased mRNA granule content, which is decreased in the *Fmr1* KO mouse [59]. Excessive AMPA receptor internalization in FMRP-deficient cultured neurons was rescued *in vitro* by MPEP administration [60]. Hippocampal slices showed normalization to wild-type values after 2 weeks of development, but not at 1 week or 8 – 10 weeks, thus emphasizing a possible importance on the timing effects of mGluR5 treatment [61]. Although MPEP does show significant reversal of phenotypic features of FXS, it is not mGluR5-specific and can inhibit the NMDA receptors at high concentrations [62] and is also toxic to humans. AFQ056 (mavoglurant) is a selective mGluR5 antagonist which reversed the inhibited startle response after prepulse and shortened elongated hippocampal neuronal spines in the mouse model of FXS [63].

Another compound demonstrating the efficacy in the mouse model is a novel, long-acting mGluR5 antagonist CTEP. Acute treatment normalized elevated hippocampal LTD, protein synthesis and susceptibility to audiogenic seizures, whereas chronic treatment with CTEP rescued cognitive deficits, auditory hypersensitivity, aberrant dendritic spine density, overactive ERK and mTOR signaling and is the first compound to partially correct macroorchidism [64].

4.2 Clinical studies

Although many studies assessing safety and efficacy of mGluR5 antagonists are currently active with pending results, some preliminary studies showed positive changes after treatment with such compounds. Fenobam was administered in a single-dose trial to 12 adults with FXS, who showed a 20% improvement in PPI and anecdotal reports of decreased anxiety [65]. Unfortunately, Neuropharm was unable to continue development due to financial constraints and an unsuccessful study of long-acting fluoxetine in ASD.

Although a randomized, double-blind, two-treatment, two-period, crossover study of AFQ056 in 30 males (18 – 35 years) [66] did not demonstrate significant treatment effects on the primary outcome measure, the Aberrant Behavior Checklist (ABC-C), an exploratory analysis showed that seven patients with full *FMR1* methylation and no detectable mRNA improved significantly on the ABC-C compared to placebo [66]. Eighteen patients with partial methylation did not show significant improvement on the medication. Overall, AFQ056 was well tolerated with mild-to-moderate side effects of fatigue and headache most commonly reported [66]. Larger, double-blind, placebo-controlled studies were completed during fall 2013 for adults (www.clinicalTrials.gov;

NCT01253629) and February 2014 for adolescents (NCT01357239), with results pending. Novartis is planning future studies for assessing the safety and efficacy in the pediatric population as well as a double-blind, placebo-controlled study of AFQ056 given in combination with a literacy intervention. Both the adult and adolescent studies are continuing with open-label extension studies (NCT01348087 and NCT01433354).

Studies assessing safety and efficacy of the mGluR5 antagonist RO4917523 (RG7090; Hoffman-La Roche) have been completed for adults and are currently active in children and adolescents (NCT01517698 and NCT01750957). Results are pending from the adult studies and open-label extension studies would be useful in assessing long-term benefits and safety. Seaside Therapeutics developed another mGluR5 antagonist, STX107, which has been successful in animal studies but will not be studied in patients with FXS due to resource limitations that forced the company to close.

5. GABA agonists

5.1 Animal studies

Since excess glutamate in the diet of the Drosophila mutant creates a toxic effect, the lethality of the *Fmr1* mutant provides an easily scored phenotype to address response to 2000 compounds [67]. Nine molecules considerably decreased the lethality and three of the nine compounds acted on GABAergic inhibitory pathways and rescued mushroom body defects, excess Futsch translation and abnormal male courtship behavior [67]. GABA dysregulation is another prominent system that is impaired in FXS and as such has strong implications for targeted treatment development to regulate inhibitory signaling and the affected pathways downstream.

The imbalance between excitation and inhibition is a key phenotypic feature of FXS and is considered the focus of both types of orphan medications. Whereas the mGluR5 antagonists' work to downregulate the overactive group 1 mGluR systems, the GABA agonists function to activate GABA transmission. GABAergic compounds have shown therapeutic efficacy in symptoms of FXS, specifically in riluzole, a medication approved for treating amyotrophic lateral sclerosis, as well as tiagabine and vigabatrin, which are FDA-approved treatments for epilepsy [68]. Arbaclofen, separate from previous GABAergic medications, focuses on the $GABA_B$ receptors and is an agonist and active enantiomer of racemic baclofen.

By upregulating the GABA system, the excessive activation in FXS can be downregulated via GABAergic medications, such as GABA agonists. Arbaclofen (STX209, R-baclofen) is a $GABA_B$ receptor agonist, which presynaptically lowers glutamate release, thereby lowering mGluR5 activation postsynaptically.

In the *Fmr1* KO mice, arbaclofen reduced the excessive upregulation of proteins resulting from the loss of FMRP that normally inhibited translation of many proteins that were important for the increased spine density of neurons and increased AMPA receptor internalization in FXS. Arbaclofen also reduced susceptibility to audiogenic seizures [69].

5.2 Clinical studies

Arbaclofen has been administered in multiple studies that assessed the safety and efficacy in pediatric, adolescent and adult patients with FXS. The first randomized, double-blind, placebo-controlled, crossover study with arbaclofen showed positive trends on the visual analog scale (VAS) and the Clinical Global Improvement Scale (CGI-I) but not on the primary outcome measure, the ABC-irritability subscale [70]. Under the modified scoring algorithm validated for FXS [71], the ABC-social avoidance subscale showed full study population improvement. Additionally, a subset of patients with significant social deficits showed improvement on all global measures and the Vineland's socialization subscale. There were few reported side effects with sedation and headache being the most common, so overall arbaclofen is a well-tolerated medication [70]. Unfortunately, limited resources prevented Seaside Therapeutics from continuing their open-label studies and the company was closed.

6. Additional targeted treatments

Despite the discontinuation of arbaclofen, there is still hope for more specific GABA agonists for FXS and other targeted treatments. Another medication developed specifically as an anticonvulsant may prove successful to target the $GABA_A$ system, which is most impaired in FXS [39,44]; Ganaxolone (3a-hydroxy-3B-methyl analog of allopregnanolone) is an orally active $GABA_A$ receptor agonist that lacks hormonal side effects. In the *Fmr1* KO mouse, ganaxolone decreased susceptibility to audiogenic seizures [72] and learning deficits may be improved through chronic activation of $GABA_A$ receptors [73]. A randomized, Phase II, double-blind, placebo-controlled, crossover trial for investigating the safety and efficacy of ganaxolone for the treatment of anxiety and attention deficits in children aged 6 – 17 years with FXS is currently under study (NCT01725152).

Another medication implicated in stabilizing inhibitory response of $GABA_A$ receptors and downstream inhibitory effects on the group 1 mGluRs is acamprosate, which is approved for treatment of alcohol withdrawal. In a small open-label study, 9 out of 12 patients between 6 and 17 years of age showed social behavior improvements and reduction in inattention/hyperactivity. A measured increase in brain-derived neurotrophic factor (BDNF) also suggests a useful pharmacodynamic marker for future acamprosate studies [74]. Randomized, double-blind, placebo-controlled studies are needed.

One pharmaceutical that has undergone considerable testing in animal models and humans is minocycline (**Figure 4**), which

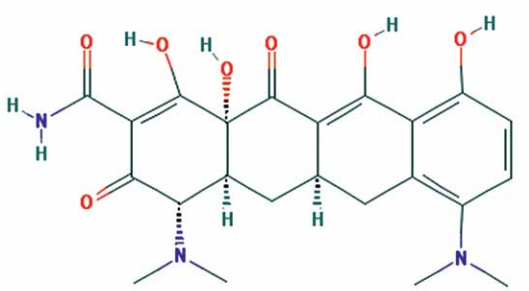

Figure 4. Illustration of minocycline.
Adapted from [96].

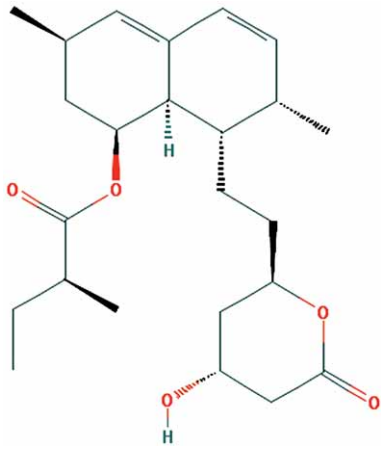

Figure 5. Illustration of lovastatin.
Adapted from [97].

is a semisynthetic tetracycline derivative antibiotic that is commonly used in treating various conditions, including Rocky Mountain Spotted Fever and acne. Dendritic spine morphology in *Fmr1* KO mice normalized, anxiety improved on the elevated plus maze and strategic exploratory behavior on the Y maze increased after treatment with minocycline [75]. Elevated MMP9 levels in the *Fmr1* KO mouse compared to wild-type were lowered after administration with minocycline [75].

MMP9 is an extracellular endopeptidase protein important for the development of synaptic connections and plasticity of the hippocampus [76]. In the Drosophila FXS model, tissue inhibitor of metalloproteinase, the only tissue inhibitor of MMPs, prevented synaptic defects, and minocycline treatment normalized dendritic spine morphology [52]. In the *Fmr1* KO mouse, minocycline administration decreased ultrasonic vocalizations during mating [77]. In 50 children treated with minocycline, 70% of families noted anecdotal improvement in language, attention and/or social interactions; however one-third of the patients experienced side effects [78]. An open-label study of minocycline in 20 boys with FXS showed improvement on the Vineland's scale and ABC-C and also found the medication to be well tolerated with the exception of two children who developed a positive antinuclear antibody (ANA) test [79]. A randomized, double-blind, placebo-controlled, crossover trial of minocycline in children with FXS, demonstrated significantly greater improvement with minocycline compared to placebo in the primary outcome, CGI-I and greater improvement in an *ad hoc* analysis of anxiety and mood-related behaviors on the VAS. Side effects were not significantly different during the minocycline and placebo treatment periods [80].

Lithium is another FDA approved medication for bipolar disorder that is often used in treating mood instability in FXS. In FXS, lithium decreases the excess of protein synthesis, and treatment with lithium in the Drosophila model of FXS rescues courtship, mushroom body defects, memory defects [54] and prevents age-dependent cognitive decline [81]. In the *Fmr1* KO mouse, lithium ameliorated hyperactivity, reversed socialization and learning deficits, normalized generalized anxiety and abnormal dendritic spine morphology [82], and reversed cognitive impairments in adolescent and adult mice [83]. An open-label trial of lithium in FXS showed significant improvements on the ABC-C total score, ABC subscores and the Vineland's adaptive behavior scale (VABS) maladaptive behavior subscore [84].

Although minocycline, lithium and lovastatin were not developed strictly for FXS, they are available clinically and may be prescribed by a patient's physician unlike the GABA agonists and mGluR5 antagonists. Numerous studies demonstrate potential benefit for patients with FXS who were treated with minocycline, and in general it is well tolerated, but side effects include GI upset, loose stools, skin sensitivity to the sun, possible darkening of the skin, gums or nails with prolonged use, rare severe headaches secondary to pseudotumor cerebri and a rare lupus like syndrome [78]. Graying of permanent teeth is also possible before permanent teeth have emerged so it is not FDA-approved for children < 8 years of age, although its use in FXS could be begun before this age with consent of the family so they are aware of the side effects. Since minocycline is an antibiotic, it will also change the gut flora, so probiotic treatment is recommended in combination along with ANA testing every 6 months.

Lovastatin (**Figure 5**), which is approved for hyperlipidemia in children and adults, has the highest transcellular permeability coefficient of statins. In mice with *Nf1*$^{+/-}$ mutation, lovastatin improved memory and long-term potentiation deficits without significant side effects [85]. Lovastatin is biologically relevant to FXS, since it acts on reducing the activation of guanosine triphosphatase Ras, leading to the activation of ERK1/2, which is a signaling molecule downstream of the mGluRs [27]. In the *Fmr1* KO mice, lovastatin decreased excessive protein production [86], blocked the induction of mGluR-mediated epileptiform activity in hippocampal slices, lowered seizures and corrected hyperexcitability in the visual cortex [87].

We have utilized lovastatin clinically in five children with FXS and have seen enhanced language responses. An 11-year-old boy with autism and FXS who was started on 20 mg/day of lovastatin was reported to have 'come out of the fog', started verbalizing more with new phrases and words, as well as improved his eye contact and overall behavior. There were no side effects and his cholesterol and other laboratory studies remained normal. A randomized, double-blind, placebo-controlled study assessing the efficacy of lovastatin treated in combination with a behavioral intervention called the Parent-Implemented Language Intervention will begin in 2015.

More compounds with planned studies in FXS include NNZ-2566 (Neuren Pharmaceuticals), which inhibits neuro-inflammation, normalizes microglia and corrects deficits in synaptic function [88] and metadoxine extended release (MG01CI; Alcobra Pharma), which has shown efficacy in treating ADHD [89]. NNZ-2566 is already under study in moderate-to-severe traumatic brain injury and Rett Syndrome with planned studies for FXS (NCT01894958). MG01CI is an ion-pair salt of pyridoxine (vitamin B6) and 2-pyrrolidone-5-carboxylate, which has dependent enzymes involved in the biosynthesis of serotonin, epinephrine, norepinephrine and GABA. It recently received orphan drug designation for the treatment of FXS, so studies are planned for 2014.

7. Expert opinion

The efficacy of reversing FXS-related phenotypes in animal models provides a promising platform for treatment in humans; however, the age sensitivity of treatment should not be ignored. Although studies do show efficacy of treatment in adult fly and mouse models, most treatments are age-dependent and can act more preventatively than correctively. That being said, the clinical studies have all started in adults, slowly going down to adolescents and then children, yet the capabilities of reversing the phenotype in fully developed brains will be far more challenging than preventing the phenotype, so the most robust efficacy is likely to be seen in the youngest patients. Due to the approval method for new drugs, studies must start assessing efficacy in adults, who are, unfortunately, more likely to show a more limited response than young children.

Outcome measures, above all, have been the most difficult component of determining efficacy. Since most patients involved in the clinical trials are patients who are more severely affected and therefore have the most room for improvement, their treatment response is measured primarily by caregiver reports such as the ABC-C edition, social responsiveness scale, repetitive behavior scale, VAS and VABS as well as the clinical global impression, which partially feeds off of caregiver report. The subjective nature of these outcome measures creates a greater demand for rigorous training of caregivers regarding how to complete these questionnaires as well as a strong explanation of the placebo effect. Caregivers of patients with FXS tend to be knowledgeable regarding the current literature and aware of the potential for these medications, but it is the role of the clinician to help the caregiver in properly differentiating between what behaviors may actually be changing as opposed to what the caregiver thinks should be changed.

The large uproar from families with children who responded well to arbaclofen and then suffered when the drug was removed as the company closed acknowledges the difficulty in finding successful outcome measures to assess efficacy. Although phenotypic improvements in animal models and human studies suggest the benefit and continued use of arbaclofen, more robust outcome measures, potential biomarkers and more adequate resources are needed to demonstrate significantly positive studies for submission to the FDA. The lack of effective outcome measures may hamper additional targeted treatment studies in patients with FXS. Biomarkers may be excellent candidates in identifying responders for specific treatments and susceptibility to side effects for FXS and other neurodevelopmental disorders. Since improvements can be subtler and occur over a longer period of time, many caregivers reported more improvements in open-label studies than during the short double-blind period and some caregivers did not fully realize the significance of the treatment until they discontinued the medication and behavioral problems returned with increased anxiety, which further advocates the necessity of a comprehensive analysis of baseline behaviors. An example of a potential biomarker is the elevated level of MMP9 in FXS, and minocycline treatment has been shown to lower the MMP9 levels [90]. However, further studies are needed to compare the lowering of this biomarker to clinical response measures. An excellent example of an outcome measure that reflects the changes in CNS function with a targeted treatment is the use of an event-related potential oddball paradigm and habituation paradigm that improved with minocycline treatment [91].

Based on anecdotal reports from caregivers in the clinical trials, the younger the participant is, the more robust and quicker the treatment seems to occur, which suggests that younger age groups are needed to prove efficacy compared to placebo. Adult studies may be unlikely to prove efficacy in a short, double-blind period due not only to the difficulty of modifying synaptic connections in a fully developed brain but also to reduced educational stimulation in the adult community. These medications are focusing on improving synaptic plasticity in order to improve behavioral phenotypes, so it is important not only to treat with the medications but also to offer education stimulation and reinforcement to maximize the effect of the medications.

Biomarkers in treatment responders are crucial to assess which patients will most likely respond or be susceptible to side effects. Despite the same FXS diagnosis, the treatment response varies considerably with some patients responding very well, whereas others have no response or a minimal response. Once effective outcome measures can be used in

correctly evaluating treatment response, then researchers will be able to determine efficacy compared to placebo. Anecdotal reports from caregivers often discuss improvements in learning (including reading), language, socialization and independence, so the AFQ056 study planned with a literacy intervention may be illuminating for improvements in cognition and learning. Although learning improvements and behavioral improvements directly impact each other, accurately gauging problematic behaviors requires report from all caregivers as well as more observational measures, whereas cognitive improvements over a longer treatment period may be easier to assess. We may be entering an age when ID will be reversed in the future which is an exciting endeavor.

At the present time, these orphan drugs do have the potential to help in treating FXS, but they are by no means a cure and caregivers should be notified of that expectation before starting so their review of behaviors will be focused more on slowly improving behavior rather than immediate response and reversal of phenotypes.

Targeted treatments are emerging in FXS and many aspects of the molecular dysfunction associated with the absence of FMRP are also seen in autism [92]. The reason for this overlap is the fact that FMRP regulates the translation of approximately 30 – 50% of the many gene messages whose mutations are associated with ASDs [21]. Therefore, it is likely that the targeted treatments for FXS, especially the mGluR5 antagonists and the GABA agonists, will also be helpful for ASD [32]. Although this was not the case with arbaclofen, this may have to do with the heterogeneity within autism. Biomarkers that will characterize those who may respond best to a GABA agonist verses an mGluR5 antagonist or minocycline will facilitate these new targeted treatment studies in both autism and in those with FXS. Currently, a few targeted treatments can be prescribed clinically, including lithium, which will downregulate the mTOR pathway [84], minocycline [80] and acamprosate [74]. In the near future ganaxolone, AFQ056 and RO4917523 will hopefully be available clinically to further improve the treatment for FXS. However, the absence or deficiency of FMRP dysregulates many pathways so that a combination of treatments is most likely to provide maximal improvement in patients with FXS [37]. In addition, learning programs for improving attention, reading and other academic areas are most likely to demonstrate improvement when combined with a targeted treatment that will enhance synaptic connections.

Declaration of interest

This work was supported by grant R40 MC 22641 from the Maternal and Child Health Research Program, Maternal and Child Health Bureau (Combating Autism Act of 2006, as amended by the Combating Autism Reauthorization Act of 2011), Health Resources and Services Administration, Department of Health and Human Services, National Institute of Health grants HD036071, DOD PR101054, support from the Health and Human Services Administration on Developmental Disabilities grant 90DD05969 and the National Center for Advancing Translational Research UL1 TR000002. RJ Hagerman and the UC Davis MIND Institute has received grants from Curemark, Forest, Novartis Pharma AG, Hoffman-La Roche, and Seaside Therapeutics to perform clinical trials in fragile X syndrome or autism. RJ Hagerman has consulted for Novartis, Roche and Genentech. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Coffee B, Keith K, Albizua I, et al. Incidence of fragile X syndrome by newborn screening for methylated FMR1 DNA. Am J Hum Genet 2009;85(4):503-14
2. Crawford DC, Acuna JM, Sherman SL. FMR1 and the fragile X syndrome: human genome epidemiology review. Genet Med 2001;3(5):359-71
3. Tassone F, Iong KP, Tong T-H, et al. FMR1 CGG allele size and prevalence ascertained through newborn screening in the united states. Genome Med 2012;4(12):100
4. Hagerman RJ, Hagerman PJ. Fragile X syndrome: diagnosis, treatment, and research. JHU Press; Baltimore: 2002
5. Roberts JE, Weisenfeld LAH, Hatton DD, et al. Social approach and autistic behavior in children with fragile X syndrome. J Autism Dev Disord 2007;37(9):1748-60
6. Sullivan K, Hatton D, Hammer J, et al. ADHD symptoms in children with FXS. Am J Med Genet A 2006;140(21):2275-88
7. Chonchaiya W, Schneider A, Hagerman RJ. Fragile X: a family of disorders. Adv Pediatr 2009;56:165-86
8. Kronk R, Bishop EE, Raspa M, et al. Prevalence, nature, and correlates of sleep problems among children with fragile X syndrome based on a large scale parent survey. Sleep 2010;33(5):679
9. Jacquemont S, Hagerman RJ, Leehey M, et al. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. Am J Hum Genet 2003;72(4):869-78
• **This paper lays out the diagnostic criteria for fragile X-associated tremor/ataxia syndrome in addition to clinical and radiological features and is of historical importance.**
10. Hagerman R, Hagerman P. Advances in clinical and molecular understanding of the fmr1 premutation and fragile X -associated tremor/ataxia syndrome. Lancet Neurol 2013;12(8):786-98
11. Sullivan SD, Welt C, Sherman S. FMR1 and the continuum of primary

ovarian insufficiency. Semin Reprod Med 2011;29(4):299-307

12. Roberts JE, Bailey DB, Mankowski J, et al. Mood and anxiety disorders in females with the FMR1 premutation. Am J Med Genet B Neuropsychiatr Genet 2009;150(1):130-9
13. Bourgeois JA, Seritan AL, Casillas EM, et al. Lifetime prevalence of mood and anxiety disorders in fragile X premutation carriers. J Clin Psychiatry 2011;72(2):175
14. Hagerman P. Fragile X -associated tremor/ataxia syndrome (FXTAS): pathology and mechanisms. Acta Neuropathol 2013;126(1):1-19
15. Hessl D, Wang JM, Schneider A, et al. Decreased fragile X mental retardation protein expression underlies amygdala dysfunction in carriers of the fragile X premutation. Biol Psychiatry 2011;70(9):859-65
16. Rodriguez-Revenga L, Madrigal I, Alegret M, et al. Evidence of depressive symptoms in fragile-X syndrome premutated females. Psychiatr Genet 2008;18(4):153-5
17. Seritan AL, Bourgeois JA, Schneider A, et al. Ages of onset of mood and anxiety disorders in fragile X premutation carriers. Curr Psychiatr Rev 2013;9(1):65-71
18. Bagni C, Tassone F, Neri G, Hagerman R. Fragile X syndrome: causes, diagnosis, mechanisms, and therapeutics. J Clin Invest 2012;122(12):4314
19. Ashley CT, Wilkinson KD, Reines D, Warren ST. FMR1 protein: conserved RNP family domains and selective rna binding. Science 1993;262(5133):563-6
20. Darnell JC, Van Driesche SJ, Zhang C, et al. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell 2011;146(2):247-61

• **This paper describes the proteins that are regulated by fragile X mental retardation protein (FMRP).**

21. Iossifov I, Ronemus M, Levy D, et al. De novo gene disruptions in children on the autistic spectrum. Neuron 2012;74(2):285-99

•• **This is a large genomics study of autism and it demonstrates that a significant portion of the genes associated with autism are linked to FMRP.**

22. Fatemi S, Folsom T, Rooney R, Thuras P. mRNA and protein expression for novel GABAA receptors θ and ρ2 are altered in schizophrenia and mood disorders; relevance to FMRP-mGluR5 signaling pathway. Transl Psychiatry 2013;3(6):e271
23. Massey PV, Bashir ZI. Long-term depression: multiple forms and implications for brain function. Trends Neurosci 2007;30(4):176-84
24. Bear MF, Huber KM, Warren ST. The mGLuR theory of fragile X mental retardation. Trends Neurosci 2004;27(7):370-7
25. Dölen G, Carpenter RL, Ocain TD, Bear MF. Mechanism-based approaches to treating fragile X. Pharmacol Ther 2010;127(1):78-93
26. Berry-Kravis E, Knox A, Hervey C. Targeted treatments for fragile X syndrome. J Neurodev Disord 2011;3(3):193-210
27. Gross C, Nakamoto M, Yao X, et al. Excess phosphoinositide 3-kinase subunit synthesis and activity as a novel therapeutic target in fragile X syndrome. J Neurosci 2010;30(32):10624-38
28. Bhattacharya A, Kaphzan H, Alvarez-Dieppa Amanda C, et al. Genetic removal of p70 S6 kinase 1 corrects molecular, synaptic, and behavioral phenotypes in fragile X syndrome mice. Neuron 2012;76(2):325-37
29. Padgett CL, Slesinger PA. GABAB receptor coupling to g-proteins and ion channels. Adv Pharmacol 2010;58:123-47
30. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci 2005;6(3):215-29
31. Cardin JA, Carlén M, Meletis K, et al. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. Nature 2009;459(7247):663-7
32. Wang LW, Berry-Kravis E, Hagerman RJ. Fragile X :leading the way for targeted treatments in autism. Neurotherapeutics 2010;7(3):264-74
33. Sohal VS, Zhang F, Yizhar O, Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature 2009;459(7247):698-702
34. Belmonte MK, Bourgeron T. Fragile X syndrome and autism at the intersection of genetic and neural networks. Nat Neurosci 2006;9(10):1221-5
35. Rubenstein J, Merzenich M. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2003;2(5):255-67
36. Berry-Kravis E, Grossman AW, Crnic LS, Greenough WT. Understanding fragile X syndrome. Curr Paediatr 2002;12(4):316-24
37. Hagerman RJ, Berry-Kravis E, Kaufmann WE, et al. Advances in the treatment of fragile X syndrome. Pediatrics 2009;123(1):378-90
38. Cordeiro L, Ballinger E, Hagerman R, Hessl D. Clinical assessment of dsm-iv anxiety disorders in fragile X syndrome: prevalence and characterization. J Neurodev Disord 2011;3(1):57-67
39. D'Hulst C, De Geest N, Reeve SP, et al. Decreased expression of the GABAA receptor in fragile X syndrome. Brain Res 2006;1121(1):238-45

• **This is one of the first papers demonstrating the GABA deficits in fragile X syndrome (FXS).**

40. lmos-Serrano JL, Paluszkiewicz SM, Martin BS, et al. Defective gabaergic neurotransmission and pharmacological rescue of neuronal hyperexcitability in the amygdala in a mouse model of fragile X syndrome. J Neurosci 2010;30(29):9929-38
41. Curia G, Papouin T, Séguéla P, Avoli M. Downregulation of tonic gabaergic inhibition in a mouse model of fragile X syndrome. Cereb Cortex 2009;19(7):1515-20
42. Adusei DC, Pacey LK, Chen D, Hampson DR. Early developmental alterations in gabaergic protein expression in fragile X knockout mice. Neuropharmacology 2010;59(3):167-71
43. Liao W-H, Van Den Abbeele T, Herman P, et al. Expression of NMDA, AMPA and GABA(A) receptor subunit mrnas in the rat auditory brainstem. II. Influence of intracochlear electrical stimulation. Hear Res 2000;150(1):12-26
44. D'Hulst C, Heulens I, Brouwer JR, et al. Expression of the gabaergic system in animal models for fragile X syndrome and fragile X associated tremor/ataxia

syndrome (FXTAS). Brain Res 2009;1253:176-83

45. Kooy FR. Of mice and the fragile X syndrome. Trends Genet 2003;19(3):148-54

46. Hagerman R, Lauterborn J, Au J, Berry-Kravis E. Fragile X syndrome and targeted treatment trials. In: Denman RB, editor, Modeling fragile X syndrome. Volume 54. Springer; Berlin Heidelberg: 2012. p. 297-335

47. Bhattacharya A, Kaphzan H, Alvarez-Dieppa AC, et al. Genetic removal of p70 S6 kinase 1 corrects molecular, synaptic, and behavioral phenotypes in fragile X syndrome mice. Neuron 2012;76(2):325-37

48. Dolen G, Osterweil E, Rao BS, et al. Correction of fragile X syndrome in mice. Neuron 2007;56(6):955-62

49. Zang JB, Nosyreva ED, Spencer CM, et al. A mouse model of the human fragile X syndrome I304n mutation. PLoS Genet 2009;5(12):e1000758

50. Dockendorff TC, Su HS, McBride SMJ, et al. Drosophila lacking dfmr1 activity show defects in circadian output and fail to maintain courtship interest. Neuron 2002;34(6):973-84

51. McBride SM, Holloway SL, Jongens TA. Using drosophila as a tool to identify pharmacological therapies for fragile X syndrome. Drug Discov Today Technol 2013;10(1):e129-36

52. Siller SS, Broadie K. Neural circuit architecture defects in a drosophila model of fragile X syndrome are alleviated by minocycline treatment and genetic removal of matrix metalloproteinase. Dis Model Mech 2011;4(5):673-85

53. Zhang YQ, Broadie K. Fathoming fragile X in fruit flies. Trends Genet 2005;21(1):37-45

54. McBride SM, Choi CH, Wang Y, et al. Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a drosophila model of fragile X syndrome. Neuron 2005;45(5):753-64

55. Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP. Suppression of two major fragile X syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. Neuropharmacology 2005;49(7):1053-66

56. de Vrij FMS, Levenga J, van der Linde HC, et al. Rescue of behavioral phenotype and neuronal protrusion morphology in fmr1 ko mice. Neurobiol Dis 2008;31(1):127-32

57. Chuang SC, Zhao W, Bauchwitz R, et al. Prolonged epileptiform discharges induced by altered group i metabotropic glutamate receptor-mediated synaptic responses in hippocampal slices of a fragile X mouse model. J Neurosci 2005;25(35):8048-55

58. Yuskaitis CJ, Mines MA, King MK, et al. Lithium ameliorates altered glycogen synthase kinase-3 and behavior in a mouse model of fragile X syndrome. Biochem Pharmacol 2010;79(4):632-46

59. Aschrafi A, Cunningham BA, Edelman GM, Vanderklish PW. The fragile X mental retardation protein and group i metabotropic glutamate receptors regulate levels of mRNA granules in brain. Proc Natl Acad Sci USA 2005;102(6):2180-5

60. Nakamoto M, Nalavadi V, Epstein MP, et al. Fragile X mental retardation protein deficiency leads to excessive mGluR5-dependent internalization of ampa receptors. Proc Natl Acad Sci USA 2007;104(39):15537-42

61. Meredith RM, de Jong R, Mansvelder HD. Functional rescue of excitatory synaptic transmission in the developing hippocampus in fmr1-ko mouse. Neurobiol Dis 2011;41(1):104-10

62. Lea PM IV, Movsesyan VA, Faden AI. Neuroprotective activity of the mGluR5 antagonists MPEP and MTEP against acute excitotoxicity differs and does not reflect actions at mGluR5 receptors. Br J Pharmacol 2005;145(4):527-34

63. Levenga J, Hayashi S, de Vrij FMS, et al. Afq056, a new mGluR5 antagonist for treatment of fragile X syndrome. Neurobiol Dis 2011;42(3):311-17

64. Michalon A, Sidorov M, Ballard TM, et al. Chronic pharmacological mGlu5 inhibition corrects fragile X in adult mice. Neuron 2012;74(1):49-56

65. Berry-Kravis E, Hessl D, Coffey S, et al. A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. J Med Genet 2009;46(4):266-71

• **The first trial of a metabotropic glutamate receptor 5 (mGluR5) antagonist, fenobam, in patients with FXS.**

66. Jacquemont S, Curie A, des Portes V, et al. Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. Sci Transl Med 2011;3(64):64ra61

•• **The first study of the mGluR5 antagonist AFQ056 in patients with FXS.**

67. Chang S, Bray SM, Li Z, et al. Identification of small molecules rescuing fragile X syndrome phenotypes in drosophila. Nat Chem Biol 2008;4(4):256-63

68. Paluszkiewicz SM, Martin BS, Huntsman MM. Fragile X syndrome: the gabaergic system and circuit dysfunction. Dev Neurosci 2011;33(5):349-64

69. Henderson C, Wijetunge L, Kinoshita MN, et al. Reversal of disease-related pathologies in the fragile X mouse model by selective activation of gabab receptors with arbaclofen. Sci Transl Med 2012;4(152):152ra128

70. Berry-Kravis EM, Hessl D, Rathmell B, et al. Effects of STX209 (arbaclofen) on neurobehavioral function in children and adults with fragile X syndrome: a randomized, controlled, phase 2 trial. Sci Transl Med 2012;4(152):152ra127

•• **The main controlled trial of arbaclofen in FXS showing the subgroups who responded well.**

71. Sansone SM, Widaman KF, Hall SS, et al. Psychometric study of the aberrant behavior checklist in fragile X syndrome and implications for targeted treatment. J Autism Dev Disord 2012;42(7):1377-92

• **Modification of the Aberrant Behavior Checklist subtest analysis to make it specific for patients with FXS.**

72. Heulens I, D'Hulst C, Van Dam D, et al. Pharmacological treatment of fragile X syndrome with gabaergic drugs in a knockout mouse model. Behav Brain Res 2012;229(1):244-9

• **Trials of GABAergic drugs to reverse symptoms in the knockout (KO) mouse.**

73. Idrissi A, Boukarrou L, Dokin C, Brown WT. Taurine improves congestive functions in a mouse model of fragile X syndrome. In: Azuma J, Schaffer S, Ito T, editors, Taurine 7. Volume 643. Springer, New York; 2009. p. 191-8

74. Erickson C, Wink L, Ray B, et al. Impact of acamprosate on behavior and

brain-derived neurotrophic factor: an open-label study in youth with fragile X syndrome. Psychopharmacology (Berl) 2013;228(1):75-84

75. Bilousova TV, Dansie L, Ngo M, et al. Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. J Med Genet 2009;46(2):94-102

76. Michaluk P, Wawrzyniak M, Alot P, et al. Influence of matrix metalloproteinase MMP-9 on dendritic spine morphology. J Cell Sci 2011;124(19):3369-80

77. Rotschafer SE, Trujillo MS, Dansie LE, et al. Minocycline treatment reverses ultrasonic vocalization production deficit in a mouse model of fragile X syndrome. Brain Res 2012;1439:7-14

78. Utari A, Chonchaiya W, Rivera SM, et al. Side effects of minocycline treatment in patients with fragile X syndrome and exploration of outcome measures. Am J Intellect Dev Disabil 2010;115(5):433-43

79. Paribello C, Tao L, Folino A, et al. Open-label add-on treatment trial of minocycline in fragile X syndrome. BMC Neurol 2010;10(1):91

80. Leigh MJ, Nguyen DV, Mu Y, et al. A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile X syndrome. J Dev Behav Pediatr 2013;34(3):147-55

•• **The first controlled trial of minocycline in children with FXS where efficacy is demonstrated.**

81. Choi CH, McBride SM, Schoenfeld BP, et al. Age-dependent cognitive impairment in a drosophila fragile X model and its pharmacological rescue. Biogerontology 2010;11(3):347-62

82. Liu ZH, Chuang DM, Smith CB. Lithium ameliorates phenotypic deficits in a mouse model of fragile X yndrome. Int J Neuropsychopharmacol 2011;4(5):618-30

83. King MK, Jope RS. Lithium treatment alleviates impaired cognition in a mouse model of fragile X syndrome. Genes Brain Behav 2013;12(7):723-31

84. Berry-Kravis E, Sumis A, Hervey C, et al. Open-label treatment trial of lithium to target the underlying defect in fragile X syndrome. J Dev Behav Pediatr 2008;29(4):293-302

85. Acosta MT, Kardel PG, Walsh KS, et al. Lovastatin as treatment for neurocognitive deficits in neurofibromatosis type 1: phase I study. Pediatr Neurol 2011;45(4):241-5

86. Osterweil EK, Krueger DD, Reinhold K, Bear MF. Hypersensitivity to mGluR5 and ERK1/2 leads to excessive protein synthesis in the hippocampus of a mouse model of fragile X syndrome. J Neurosci 2010;30(46):15616-27

87. Osterweil EK, Chuang S-C, Chubykin AA, et al. Lovastatin corrects excess protein synthesis and prevents epileptogenesis in a mouse model of fragile X syndrome. Neuron 2013;77(2):243-50

•• **This paper demonstrates that lovastatin can rescue the seizure phenotype and excess protein synthesis in the KO mouse.**

88. Cartagena CM, Phillips KL, Williams GL, et al. Mechanism of action for NNZ-2566 anti-inflammatory effects following PBBI involves upregulation of immunomodulator ATF3. Neuromolecular Med 2013;15(3):504-14

89. Manor I, Newcorn JH, Faraone SV, Adler LA. Efficacy of metadoxine extended release in patients with predominantly inattentive subtype attention-deficit/hyperactivity disorder. Postgrad Med 2013;125(4):181-90

90. Dziembowska M, Pretto DI, Janusz A, et al. High MMP-9 activity levels in fragile X syndrome are lowered by minocycline. Am J Med Genet A 2013;161a(8):1897-903

• **This paper suggests that MMP9 levels may be a good biomarker for minocycline treatment of FXS.**

91. Schneider A, Leigh MJ, Adams P, et al. Electrocortical changes associated with minocycline treatment in fragile X syndrome. J Psychopharmacol 2013;27(10):956-63

• **This paper uses an event- related potential paradigm to demonstrate improvement in brain processing and habituation in patients with FXS treated with minocycline.**

92. Hagerman R, Au J, Hagerman P. FMR1 premutation and full mutation molecular mechanisms related to autism. J Neurodev Disord 2011;3(3):211-24

93. National Center for Biotechnology Information. PubChem Compound Database; CID=9926832. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=9926832 [Accessed 15 December 2013]

94. National Center for Biotechnology Information. PubChem Compound Database; CID= 44602. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=44602 [Accessed 15 December 2013]

95. National Center for Biotechnology Information. PubChem Compound Database; CID= 6918305. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6918305 [Accessed 15 December 2013]

96. National Center for Biotechnology Information. PubChem Compound Database; CID=54675783. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=54675783 [Accessed 15 December 2013]

97. National Center for Biotechnology Information. PubChem Compound Database; CID=53232. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=53232 [Accessed 15 December 2013]

98. Berry-Kravis E, Krause SE, Block SS, et al. Effect of CX516, an AMPA-modulating compound, on cognition and behavior in fragile X syndrome: a controlled trial. J Child Adolesc Psychopharmacol 2006;16(5):525-40

Affiliation

Emma B Hare[1] BA, Randi J Hagerman[2] MD & Reymundo Lozano[†3] MD
[†]Author for correspondence
[1]Certified Clinical Research Coordinator, UC Davis MIND Institute, 2825 50th Street, Sacramento, CA 95817, USA
[2]Medical Director of the MIND Institute, UC Davis MIND Institute, 2825 50th Street, Sacramento, CA 95817, USA
[3]UC Davis MIND Institute, 2825 50th Street, Sacramento, CA 95817, USA
Tel: +1 916 703 0494;
Fax: +1 916 703 0240;
E-mail: reymundo.lozano@ucdmc.ucdavis.edu

CHAPTER 9

Treatment of Fragile X Syndrome and Fragile X-associated Disorders

REYMUNDO LOZANO, EMMA B. HARE, AND RANDI JENSSEN HAGERMAN

INTRODUCTION

Fragile X syndrome (FXS) and fragile X-associated disorders (FADs) are all related to mutations in the fragile X mental retardation 1 gene (*FMR1*) on the bottom end of the X chromosome at Xq27.3 position. In the normal range, there are 5 to 40 CGG repeats in the 5' untranslated region of *FMR1*. In carriers of the premutation, there are 55 to 200 CGG repeats, and in the full mutation there are more than 200 repeats that are usually methylated. In the full mutation, methylation shuts down transcription, and the lack or deficiency of the *FMR1* protein (FMRP) causes FXS. The premutation leads to the FADs because there is enhanced transcription leading to excessive *FMR1* mRNA and RNA toxicity (Tassone et al., 2000; Hagerman & Hagerman, 2013). The high end of the premutation can lead to lowered FMRP levels and mild symptoms that are typical in FXS (Goodlin-Jones et al., 2004). The RNA toxicity, however, can lead to emotional difficulties in childhood, including ADHD, autism spectrum disorders (ASD), shyness, and social anxiety (Farzin et al., 2006; Chonchiaya et al., 2012). These problems can persist into adulthood, although depression and anxiety are the most common difficulties of adults with the premutation and normal intellectual abilities (Bourgeois et al., 2011; Hagerman & Hagerman 2013).

The premutation is the most common genetic cause of primary ovarian insufficiency (FXPOI), meaning cessation of menses before age 40,

and approximately 20% of carriers experience this problem (Sullivan et al., 2011). Approximately 54% of female and 27% of male premutation carriers experience migraine headaches (Au et al., 2013); the majority of carriers develop hypertension with age (Hamlin et al., 2012); and immune-mediated problems such as fibromyalgia and hypothyroidism are common in women who are carriers (Winarni et al., 2012a). Neurological problems are seen in aging premutation carriers, including neuropathy, tremor, ataxia, and cognitive decline, all symptoms of the fragile X-associated tremor ataxia syndrome (FXTAS). These neurological problems occur in approximately 40% of male carriers and 16% of female carriers, although the symptoms in females are less severe and dementia is rare (Hagerman & Hagerman, 2013).

This chapter will focus on targeted treatments developed for FXS, because recent progress in understanding the biological mechanisms of the disorder have led to the development of several medications that are leading the way for targeted treatments, not only in FXS, but also in related disorders, including autism and ASD. Some of the targeted treatments developed for FXS may also be helpful for premutation disorders (FAD), but additional research is needed before validating these treatments in carriers (Cao et al., 2012).

FXS is the most common inherited cause of intellectual disability (ID) and the most common single gene cause of autism known. The prevalence of the premutation in the general population is approximately 1 in 200 females and 1 in 450 males (Tassone et al., 2012), whereas the prevalence of the full mutation is approximately 1 in 4,000 males and females (Crawford et al., 2001).

CLINICAL FEATURES

Those with FXS usually do not have dysmorphic features, although the ears may be prominent with cupping in the upper aspect of the pinna, and the face may be long, but typically not until after adolescence (Figure 9.1). The skin is usually soft, and the finger joints may be hyperextensible in childhood because of loose connective tissue. The feet are generally flat with some degree of pronation, and the testicles become large in adolescence and then stabilize in size, such that they are two to three times larger than in normal males (Hagerman & Hagerman, 2002). A high arched palate is common, and this may influence the drainage of the Eustachian tubes. Ear infections are common in the first three to four years of life, and pressure equalizing (PE) tubes are often used to normalize hearing and improve

Figure 9.1:
Two boys with FXS. One displays typical fragile X features, specifically prominent ears, while the other does not.

language. Seizures occur in approximately 20% of children with FXS and up to 13% of children with the premutation, and anticonvulsants are an effective treatment (Hagerman & Hagerman 2002; Chonchiaya et al., 2012). The presence of seizures is associated with a higher risk for ASD, so treatment is essential (Chonchiaya et al., 2012).

Because most children with FXS look normal, the key to their diagnosis is in their behavior. By the second year of life, they develop increasing anxiety and hypersensitivity to sensory stimuli. Hyperactivity is seen in most boys and in up to 50% of girls with the full mutation, although attention and executive function deficits are common even when they are not hyperactive (Cornish et al., 2013). Infants are often hypotonic in the first and second year of life and delayed in language, typically not speaking at age two. Additional behavior problems include poor eye contact and hand-flapping with excitement, along with perseveration in behavior and tantrums. Autism is diagnosed in 30% of boys with FXS and an ASD is seen in up to 60% (Harris et al., 2008). The children with an ASD demonstrate significant social and language deficits beyond just poor eye contact or repetitive behaviors (Kaufmann et al., 2004) (Table 9.1).

Typically, children with FXS are diagnosed around three years of age with *FMR1* DNA testing (Bailey et al., 2009). High-functioning boys with an IQ greater than 70 and girls with FXS who typically present with learning

Table 9.1 CLINICAL CHARACTERISTICS OF FRAGILE X-ASSOCIATED DISORDERS

Premutation (55–200 CGG repeats)	Full Mutation (>200 CGG repeats)
Physical/Medical features (less frequent than in the full mutation):	*Physical/Medical features:*
• Prominent ears	• Prominent ears with cupping
• Hyperextensible finger joints	• Elongated face after adolescence
• Seizures (8–13%)	• Hyperextensible joints
• Migraines	• Loose connective tissue
• Immune mediated disorders	• Soft skin
• Fibromyalgia	• Flat feet with some pronation
• Hypothyroidism	• Macroorchidism after age 10
• Primary ovarian insufficiency (FXPOI)	• High arched palate
• Aging: Fragile X-associated tremor/ataxia syndrome (FXTAS)	• Seizures (20%)
• Neuropathy	
• Tremor	
• Ataxia	
• Cognitive decline	
• Executive function deficits	
Cognitive features:	*Cognitive features*:
• Social anxiety	• ADHD
• Depression	• Intellectual disability
• Anxiety	• Autism
• ADHD	• Autism spectrum disorders (ASD)
• Shyness	• Anxiety
• Autism spectrum disorders (ASD)	• Hypersensitivity to sensory stimuli
• Executive function deficits	• Executive function deficits
	• Social anxiety
	• Poor eye contact
	• Hand flapping
	• Perseveration
	• Tantrums
	• Social and language deficits
	• Learning disabilities
Prevalence:	*Prevalence:*
• 1 in 200 females	• 1 in 4,000 males and females
• 1 in 450 males	

disabilities, such as math problems, and anxiety, but without ID, are often not diagnosed until later in childhood or even adolescence. On occasion, a grandfather is diagnosed with FXTAS or a mother is diagnosed with FXPOI, which precipitates cascade testing of other family members, leading to diagnosis of a child with FXS or another family member with FAD.

The average IQ of an adult male with FXS is 40, but those with a lack or partial lack of methylation (methylation mosaicism) or those who have size mosaicism (some cells with the premutation and other cells with the full mutation) have an average IQ in the 60s (Hagerman & Hagerman, 2002). Approximately 15% of boys with FXS are high-functioning with an IQ greater than 70. Most girls with FXS, on the other hand, have an IQ above 70, although 25% have ID. The X-activation ratio, meaning the percentage of cells with the normal X as the active X, correlates with the overall IQ in girls with FXS (Loesch et al., 2004).

NEUROBIOLOGICAL MECHANISMS OF DISEASE

Over two decades of molecular research have led to significant advances in understanding the neurobiology of FXS and related disorders. FMRP is a selective RNA-binding protein, which regulates the translation of hundreds of mRNAs, usually through inhibition (Bagni et al., 2012; Darnell & Klann, 2013). FMRP contains three main RNA-binding domains; two hnRNP K-homology (KH) domains and one RGG box. In addition, a stem loop SoSLIP motif and U-rich sequences have been proposed to be RNA-binding sites. The I304N point mutation, which is located within the second KH domain, causes severe FXS and suggests that this domain plays an essential role in the FMRP function. FMRP is largely found in the cytoplasm; however, it contains a nuclear localization and nuclear export sequence. FMRP regulates RNA transportation, stabilization, and translation. *In vitro* FMRP is part of messenger ribonucleoparticles (structures that are involved in protein synthesis) and regulates dendritic transport of associated mRNAs, which result in the production of protein synthesis at the synapse (Bagni et al., 2012). FMRP interacts with several cytoplasmic and nuclear proteins, and it has been found in granules containing translationally silent preinitiation complexes. It is estimated that FMRP binds about 4% of total brain RNA and interacts with many other proteins, including approximately 30% of the proteins associated with autism (Bagni et al., 2012; Darnell et al., 2011, 2013; Iossifov et al., 2012).

In the brain, protein synthesis in the soma, axons, dendrites, and postsynaptic sites is required for long-term forms of synaptic plasticity, which

form and consolidate long-term memories. Protein synthesis promotes synaptic plasticity activation, as well as the activation of different synaptic plasticity states, and it is coordinated by the action of the metabotrobic glutamate receptors (mGluRs) (Massey & Bashir 2007). Specifically, the activation of mGluR induces a synaptic plasticity state called "long-term depression" (LTD) (Massey & Bashir 2007), which triggers synaptic plasticity by regulating mRNA and the synthesis, degradation and recycling of somatic and axonic proteins. In the *Fmr1*-KO mice, LTD is significantly increased (Bagni et al., 2012). This effect on LTD is probably due to dysregulated local protein synthesis and has established the basis of the "mGluR theory" (Bear et al., 2004). The mGluR theory of FXS suggests that the psychiatric, cognitive, and neurological aspects of the syndrome are due to exaggerated downstream consequences of mGluR5 upregulation. This theory was validated by genetic mouse studies where rescue of several symptoms occurred when the mGluR5 heterozygous mouse was crossed with the *Fmr1*-KO mouse (Dolen et al., 2010). The *Fmr1*-KO shows an excess of protein translation, protein synthesis, and synaptic proteins (Berry-Kravis et al., 2011; Bagni et al., 2012). Additionally, FMRP binds and represses the catalytic subunit of PI3K, a signaling molecule downstream of the activation of mGluR5 (Gross et al., 2010). In summary, the absence of FMRP leads to dysregulation and usually over-expression of a number of its target genes, which causes abnormal synthesis of proteins involved in neurotransmission, dendritic morphology, and synaptic plasticity. Several approaches, including the use of mGluR5 antagonists, have led to positive outcomes for anatomical, electrophysiological, and behavioral measures in the animal model, leading to subsequent human trials (Hagerman et al., 2012) (Figure 9.2). Targeted treatments show promising results in mitigating or even reversing the neurobiological abnormalities caused by loss of FMRP. Furthermore, targeted treatments for FXS are leading the way for treatment of other neurodevelopmental disorders, including autism and ASD.

DIAGNOSTIC METHODS

The diagnosis of FXS or the premutation is made with molecular testing for the cytosine, guanine, guanine (CGG) expansion in *FMR1* (order the *FMR1* DNA test) that includes polymerase chain reaction (PCR) and Southern Blotting. The latter is most important to see if a large expansion in the full mutation range is present and to document the methylation status. PCR demonstrates the size of the premutation and the genetic report should

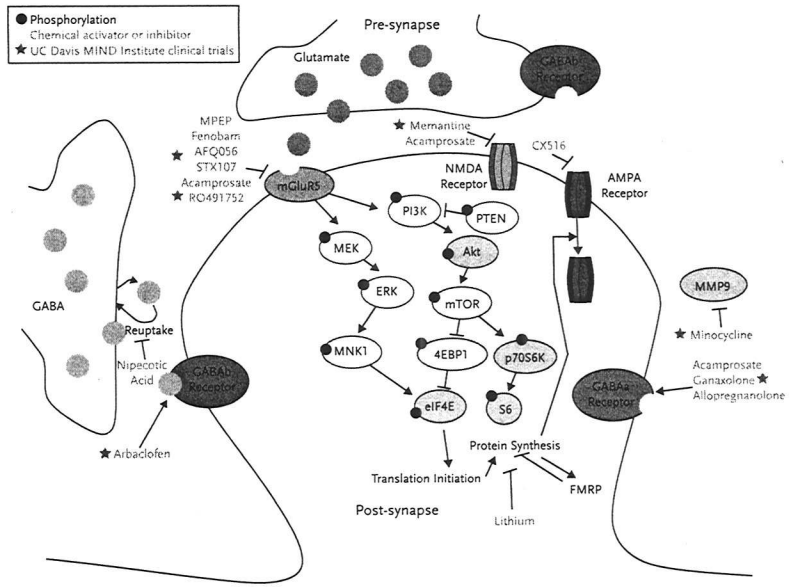

Figure 9.2:
Diagram of mechanism implicated in FXS altered synaptic plasticity and targeted treatments. Two signaling pathways downstream of mGluR5 affect translation, the MEK-ERK-Mnk1 and the PI3K-mTOR pathway. Also depicted are medications that affect the GABA receptors, MMP9 level, NMDA, and AMPA receptors. The stars represent clinical trials at the MIND Institute. Picture adapted from Levenga et al., 2010, and artistic input from Carolyn Yrigollen and Dr. Flora Tassone.

document the size of the CGG repeat. All children or adults diagnosed with ASD or ID of unknown etiology should be tested for FXS with the *FMR1* DNA testing. Those with neurological problems, including tremor or ataxia and women with POI, should also have *FMR1* DNA testing, and this can be ordered by any physician, usually the primary health care provider.

RELATED DISORDERS

There is a close association between ASD and FXS because FMRP regulates hundreds of genes at the synapse that are important for synaptic plasticity, and many of these genes are also associated with autism, including neuroligins, neurexins, *PSD 95, Shank 3, Arc*, etc. (Iossifov et al., 2012; Darnell & Klann, 2013). Therefore the lack of FMRP in FXS will lead to dysfunction of these other genes associated with autism. Low levels of FMRP in the CNS are also seen in autism without an *FMR1* mutation (Fatimi et al., 2011) and in other psychiatric conditions including schizophrenia, bipolar disorder,

and depression (Fatimi et al., 2010). Recent studies have shown that FMRP levels in blood are associated with the age of onset and IQ in those with schizophrenia but without an *FMR1* mutation (Kovacs et al., 2013).

The gray zone includes those with 40 to 54 CGG repeats and it is called gray because it is unclear if disease is associated with this allele size. There is twice the rate of FXPOI in the gray zone than in the general population (Bretherick et al., 2005) and this is likely to be related to a mild elevation of *FMR1* mRNA levels (Loesch et al., 2007). In addition, FXTAS has now been diagnosed in the gray zone (Hall et al., 2011), and this is perhaps more common when others in the family have FXTAS (Liu et al., 2012).

CURRENT STANDARD TREATMENT OF FXS

Currently, early intervention with both speech and language therapy and occupational therapy with sensory integration techniques are given to children with FXS, most optimally beginning in the first year of life, but more typically obtained after diagnosis is made. Most children benefit significantly from special education support, but whenever possible, mainstreaming them into the regular classroom with the support of an aide is helpful (Hagerman & Hagerman, 2002). Enrichment of the environment is essential for an optimal outcome, and home intervention for early language development and motor development should start in the first year. For those with autism, the Early Start Denver Model (ESDM) developed by Sally Rogers and Geri Dawson is recommended (Dawson et al., 2010).

Many medications can be helpful in children with FXS, and the use of sertraline, a selective serotonin reuptake inhibitor (SSRI), may enhance language development at low doses beginning at age two (Winarni et al., 2012 a and b). However, hyperarousal can be seen in 20% of those treated with sertraline, and if activation or an increase in aggression occurs with sertraline, the dose should be lowered or discontinued. Typically sertraline or another SSRI can also decrease anxiety, and this may improve behavior and social interactions, which anxiety can worsen. The use of aripiprazole (Abilify) or risperidone (Risperdal) can help to stabilize mood, decrease aggression, and improve anxiety (Hagerman et al., 2009; Erickson et al., 2011). Clonidine or guanfacine can also have a calming effect in children with FXS and reduce hyperarousal, aggression, or hyperactivity. Clonidine can also help with sleep, although melatonin should be the first treatment for the sleep disturbances that are seen in the majority of young children with FXS (Wirojanan et al., 2009). Stimulants are helpful to treat ADHD symptoms in children with FXS who are five or older and can also be helpful throughout adolescence (Hagerman et al., 2009).

Currently, many of the targeted treatments outlined below are often mixed with standard treatments outlined above for an optimal effect for the child or adult with FXS.

TARGETED TREATMENTS

mGluR5 Antagonists

Since upregulation of the mGluR5 system leads to LTD, pharmaceutical interventions to downregulate mGluR5 activity have been shown to correct aspects of the FXS phenotype (Hagerman et al., 2012). There is a heightened focus on the development of mGluR5 negative allosteric modulators, resulting in the study of multiple compounds, including MPEP (2-methyl-6-phenylethynyl pyridine hydrochloride), fenobam (Neuropharm Ltd.), AFQ056 (Mavoglurant; Novartis Pharmaceuticals), RO4917523 (Hoffman-La Roche), and STX107 (Seaside Therapeutics).

Studies addressing MPEP treatment in *Fmr1*-KO mice have rescued an array of phenotypes typical of FXS, including correction of increased prepulse inhibition, audiogenic seizures, enhanced locomotor activity in the open field, increased density, and weak or immature synaptic architecture (reviewed in Hagerman et al., 2012). *In vitro* MPEP additionally rescued excessive AMPA receptor internalization in FMRP-deficient cultured neurons (Nakamoto et al., 2007). Of consideration, however, is the timing of mGluR5 antagonist treatment. When studying the effects of mGluR5 blockade on excitatory synaptic activity, normalization to wild-type values was only evident in hippocampal slices after two weeks of development, but not at one week or eight to ten weeks (Meredith et al., 2011). Chronic treatment with CTEP, another mGluR5 antagonist, corrected elevated hippocampal LTD, enhanced protein synthesis, audiogenic seizures, auditory hypersensitivity, irregular dendritic spine density, overactive ERK, and mTOR signaling, and partially corrected macroorchidism in young adult KO mice (Michalon et al., 2012). This study showed that pharmacological treatment after mature development can reverse many aspects of the FXS phenotype, and this has given hope to many families that have an adult with FXS.

Two genetic investigations of mGluR5 regulation in *Fmr1*-KO mice that reduced FMRP levels by 50% presented contradicting results of decreasing susceptibility to audiogenic seizures. While both studies presented the rescue of numerous phenotypes, reduced susceptibility to audiogenic seizures

was only found in one study (Dolen et al., 2007), while the other reported no significant rescue of susceptibility to seizures (Thomas et al., 2012). This contradicting result demonstrates need for continued study and reinforces the consideration of factors such as background genetic effects or environmental factors that may contribute to the impact of treatment. Given the overall rescue of numerous phenotypical features of FXS in animal models, multiple mGluR5 antagonists are now under study in humans, with promising outcomes.

Human Studies

In a single-dose trial with fenobam administered to 12 adults with FXS, subjects showed a 20% improvement in prepulse inhibition (PPI), which involves sensorimotor gating and inhibition (Berry-Kravis et al., 2009). Due to financial constraints, however, Neuropharm was unable to pursue further development despite encouraging results.

The second clinical study with an mGluR5 antagonist was a randomized, double-blind, two-treatment, two-period, crossover study of AFQ056 in 30 males (18–35 years) (Jacquemont et al., 2011). While the primary outcome measure, the Aberrant Behavior Checklist–Community Edition (ABC-C), did not demonstrate significant treatment effects, an exploratory analysis showed seven patients with full *FMR1* methylation and no detectable *FMR1* messenger RNA improved significantly on the ABC-C compared to placebo. Eighteen patients with partial methylation showed a variable response, but overall there was no significant improvement on the medication. AFQ056 was well tolerated, with mild to moderate fatigue and headache the most commonly reported side effects (Jacquemont et al., 2011). Novartis is pursuing global, randomized, double-blind, placebo-controlled studies to evaluate the safety and efficacy of AFQ056 in children, adolescents, and adults with FXS (see the website clinicaltrials.gov for further information). In addition, open-label continuation studies are ongoing for adults and adolescents with FXS. Because there has been a mixed response to this medication, testing for biomarkers that could identify the responders, such as complete methylation of *FMR1*, is taking place.

Studies assessing efficacy of the mGluR5 antagonist RO4917523 (Hoffman-La Roche) are currently underway in children beginning at age five and into adulthood with FXS. These studies are at multiple centers internationally and are controlled trials, although open-label longitudinal studies have not yet been organized. If efficacy is seen in these studies, then phase 3 trials will be organized. Seaside Therapeutics, which recently partnered with Hoffman-La Roche, has developed another mGluR5 antagonist, STX107, which has been successful in animal studies and will be studied in patients with FXS in the future (Figure 9.2).

TARGETING $GABA_A$ RECEPTORS

In FXS, collective results show an imbalance between neuronal inhibition and excitation with overall excitation; therefore, changing the balance from excitation to inhibition has been considered through a GABAergic approach. The gamma amino-butyric acid (GABA) pathways are the main inhibitory system in the human brain and play a role in regulating neuronal excitability throughout the nervous system. There are two classes of GABA receptors: $GABA_A$ and $GABA_B$. $GABA_A$ receptors are ligand-gated ion channels, whereas $GABA_B$ receptors are G protein-coupled receptors. $GABA_A$ receptors allow the flow of chloride ions across the membrane, which hyperpolarizes the neuron's membrane and minimizes the effect of any coincident synaptic input. FMRP targets the mRNAs encoding eight different $GABA_A$ receptor subunits ($\alpha1$, $\alpha3$, $\alpha4$, $\beta1$, $\beta2$, $\gamma1$, $\gamma2$, and χ), which were significantly reduced in the cortex of *Fmr1*-KO mice, particularly the γ subunit, which is believed to represent extrasynaptic (perisynaptic) $GABA_A$ receptors (D'Hulst et al., 2006). In addition, the *Fmr1*-KO mouse exhibits reduced inhibitory postsynaptic currents in the amygdala (Olmos-Serrano et al., 2010) and subicular neurons (Curia et al., 2009). Fly models of FXS also show lower levels of GABA receptors (Chang et al., 2008). $GABA_A$ agonists act to directly compensate for the $GABA_A$ subunit deficiencies by enhancing the function of the existing receptors.

A brief report of three patients with FXS and autism treated with acamprosate showed improvement in language and behavior in all the patients (Erickson et al., 2010). Acamprosate is a drug approved for treatment of alcohol withdrawal; it is thought to stabilize the excitatory/inhibition balance in the brain mainly by enhancing the function of $GABA_A$ receptors and possibly inhibitory effects at group I mGluRs (Erickson et al., 2010).

Ganaxolone (3a-hydroxy-3B-methyl analogue of allopregnanolone) is a $GABA_A$ receptor agonist through allosteric modulation; it is orally active and lacks hormonal side effects (Pin & Prézeau, 2007). In the *Fmr1*-KO, ganaxolone has been shown to decrease audiogenic seizures (Heulens et al., 2012). Similarly, studies in the *dfmr* mutant fly show that $GABA_A$ agonists ameliorate the lethality phenotype from glutamate-containing food, neuropathology, excessive protein translation, and abnormal courtship behavior (Chang et al., 2008). The chronic activation of $GABA_A$ receptors may

have beneficial effects in ameliorating the learning deficits characteristic of the FXS (El Idrissi et al., 2009). Currently we are conducting a randomized, phase 2, double-blind, placebo-controlled crossover trial to investigate the efficacy of ganaxolone for the treatment of anxiety and attention deficits in children with FXS ages 6 to 17 (www.clinicaltrials.org).

TARGETING $GABA_B$ RECEPTORS

Arbaclofen (STX209, R-baclofen) is a γ-aminobutyric acid type B ($GABA_B$) receptor agonist and the active enantiomer of racemic baclofen, which acts presynaptically to block glutamate release. The resulting decreased glutamatergic drive indirectly reduces mGluR5 activation (Figure 9.2). In addition to exaggerated mGluR1/5 activation in FXS explained above is the deficiency of GABA-mediated inhibitory neurotransmission. *Fmr1*-KO mice show reduced GABAergic inhibition in the hippocampus, striatum, somatosensory cortex, and amygdala (Olmos-Serrano et al., 2010). Humans with FXS are shown to have excessive amgydala activation, as is evident during face-processing tasks (Watson et al., 2009). However, another recent study suggested the opposite; lower FMRP levels in fragile X spectrum involvement (FXSI) from the premutation to the full mutation were associated with lowered activation of the amygdala on fMRI studies (Kim et al., 2014). Dysregulation of the GABA pathways in the limbic system are hypothesized to be the basis of social anxiety and avoidance, characteristic of FXS (Cordiero et al., 2011). In the *Fmr1*-KO mice, arbaclofen reduced protein synthesis and translation to wild-type levels, corrected AMPAR trafficking in neurons, corrected the increased spine density, and rescued susceptibility to audiogenic seizures (Henderson et al., 2012). Therefore arbaclofen is considered a disease-modifying drug or targeted treatment for FXS.

Human Studies

A randomized, double-blind, placebo-controlled crossover study with arbaclofen evaluating improvements in behavioral symptoms of 63 subjects with FXS (55 males) aged 6–40 years showed significant treatment effects on numerous outcome measures (Berry-Kravis et al., 2012). While the primary outcome measure, the ABC-Irritability subscale, did not show significance, parent-nominated problem behaviors on the Visual Analog Scale (VAS) and the Clinical Global Improvement scale (CGI-I) showed positive trends with treatment of arbaclofen. Additional post hoc analysis demonstrated full study population improvement on the ABC-Social Avoidance (SA) subscale validated for FXS (Sansone et al., 2012), which is currently being used as an efficacy assessment in multiple clinical trials for FXS. Furthermore, a subset of patients who met diagnostic criteria for autism or had significant social deficits on the ABC (27 subjects) demonstrated additional improvements in the Vineland-Socialization subscale and all global measures. Overall, arbaclofen is a well-tolerated medication with few side effects, the most common being sedation and headache in only 8% (Berry-Kravis et al., 2012). Many of the subjects continued to extension studies. However, further controlled studies in those with ASD and in those with FXS did not show significant efficacy, leading to the collapse of the company and the termination of all studies.

LOVASTATIN

Lovastatin was originally isolated from the mold Aspergillus and it is also naturally found in the culinary oyster mushroom. It was the first statin utilized clinically, and it has the greatest transcellular permeability coefficient; therefore, it reaches the highest central nervous system (CNS) levels when compared to other statins. Lovastatin is a specific inhibitor of the rate-limiting enzyme in cholesterol biosynthesis (3-hydroxy-3-methylglutaryl coenzyme A [3HMG-CoA] reductase), and it is widely used for treatment of hyperlipidemia in children and adults (Figure 9.2). Although lovastatin is FDA-approved for use in children 10 years and older for treatment of familial hypercholesterolemia, it has been used in younger children for other conditions, such as cholesterol ester storage disease and nephrotic syndrome (Prata et al., 1994). Most recently, lovastatin has been used successfully in infants to treat hypoxia and ischemic encephalopathy (Buonocore et al., 2012). Lovastatin is also a targeted treatment for neurofibromatosis type 1 (NF1) because it can inhibit small GTPases (including Ras) (Li et al., 2007). In mice with *Nf1*+/– mutation, lovastatin improved memory and long-term deficits in potentiation without significant side effects (Acosta et al., 2011).

Pertinent to FXS, lovastatin reduces the activation of the small guanosine triphosphatase (GTPase) Ras and subsequently the activation of the extracellular signal regulated kinase (ERK1/2), a signaling molecule downstream to the activation of mGluRs (Gross et al., 2010). Specifically, lovastatin interferes with recruitment of Ras to the membrane, a process required to transition from inactive GDP to active GTP. The interaction of Ras with the membrane requires the post-translational addition of a farnesyl group to

the C terminus of Ras. Lovastatin inhibits Ras farnesylation by targeting the upstream mevalonate pathway (Osterweil et al., 2010).

In the *Fmr1*-KO, lovastatin decreased the excessive protein production (Osterweil et al., 2010) by inhibition of the Ras-ERK1/2 signaling in the hippocampal neurons (Li et al., 2007). In addition, the use of lovastatin blocked the induction of mGluR-mediated epileptiform activity in hippocampal slices, lowered seizures, and corrected hyperexcitability in visual cortex in the *Fmr1*-KO (Osterweil et al., 2013).

We have utilized lovastatin clinically in five children with FXS, and we have seen a response in enhanced language. For instance, the mother of an 11-year-old old boy with autism and FXS who was started on lovastatin (20 mg a day) stated that he "came out of the fog"; he started verbalizing more with the utilization of phrases for the first time and "using more combinations of new words." His eye contact improved, as did his overall behavior. Although his mother states that he is still "spinning things," she adds that "he is now using pretend play, which is new for him." There were no side effects, and his cholesterol and other laboratory studies remain normal. However, these are anecdotal reports and there is a need for a controlled trial of lovastatin to assess its safety and efficacy in children with FXS.

MINOCYCLINE

Animal Studies

When Bilousova et al. (2009) published the first *fmr1*-KO mouse studies utilizing minocycline, the fragile X field was surprised that one month of minocycline after birth could rescue the dendritic spine defects seen in FXS. The long, thin and immature spines converted to normal mature spines on minocycline, and there were improvements in anxiety on the elevated plus maze along with improvements on a cognitive task (Bilousova et al., 2009). These researchers found elevated matrix metalloproteinase 9 (MMP9) levels in the *fmr1*-KO mouse compared to wild type and minocycline lowered the MMP9 levels to normal (Figure 9.2).

MMP9 is an endopeptidase that is extracellular, but it also appears to be an important protein for the development of synaptic connections and plasticity, particularly in the hippocampus (Michaluk et al., 2011). In the *Drosophila* model of fragile X (*dfmr1* mutants), Kendall Broadie's laboratory demonstrated that over-expression of the only tissue inhibitor of MMPs, tissue inhibitor of metalloproteinase (TIMP), prevented the synaptic defects seen in the *dfmr1* mutants (Siller & Broadie, 2011). Minocycline treatment of the *dfmr1* mutants normalized synaptic structure and brain morphology (Siller & Broadie 2011). Subsequently, Rotschafer et al. (2011) have demonstrated decreased ultrasonic calling vocalizations during mating in the *fmr1*-KO mouse compared to wild type, and treatment for four weeks after birth with minocycline normalized the deficient vocalizations. These animal studies have paved the way for clinical trials with minocycline in patients with FXS.

Human Studies

Minocycline is a semisynthetic tetracycline-derivative antibiotic that has been available since the 1960s and is a common treatment for multiple conditions, including Rocky Mountain spotted fever and acne vulgaris. The initial human studies in FXS involved a survey of the families whose children with FXS were treated clinically with minocycline after the Bilousova study was published. Utari et al. (2010) utilized a Likert scale to survey the parents of 50 children treated for at least two weeks and up to a year and found that 70% of families noted improvement in language, attention, and/or social interactions, although side effects occurred in over one-third of patients. Paribello et al. (2010) carried out an open-label add-on-study of minocycline in 20 males with FXS who were 13 to 32 years of age and found improvement in a variety of measures, including the Vineland and the ABC scale. They found minocycline to be well tolerated, but 2 of the 20 patients treated developed a positive antinuclear antibody (ANA) test.

Due to these initial positive responses, Leigh et al. initiated a randomized, double-blind, placebo-controlled, crossover trial in individuals with FXS, ages 3.5–16 years (n = 55, mean age 9.2, SD 3.6 years) (Leigh et al., 2013). Participants were first randomized to three months of minocycline or placebo, and then switched to the other treatment arm for an additional three months. Primary outcome measures were the Clinical Global Impressions Scale–Improvement (CGI-I) and the Visual Analogue Scale (VAS) for behavior difficulties. Sixty-nine subjects were screened and 66 were randomized. Fifty-five subjects (83.3%) completed at least the first period, and 48 (72.7%) completed the full trial. The results demonstrated, in an intention-to-treat analysis, significantly greater improvement (lower score) in the primary outcome, CGI-I, after minocycline compared to placebo (least squares means ± standard error: 2.49 ± 0.13, 2.97 ± 0.13, respectively, p 0.0173) and greater improvement (higher number) in an ad hoc analysis of anxiety and mood-related behaviors on the VAS (minocycline 5.26 cm ± 0.46 cm, placebo 4.05 cm ± 0.46 cm; p 0.0488). The secondary outcome measures, including the ABC, the Vineland Adaptive Behavior

Scales (2nd edition), and the Expressive Vocabulary Test (EVT), had no significant improvement on minocycline. Side effects were not significantly different during the minocycline and placebo treatment periods. No serious adverse events occurred during minocycline treatment even in the young children at 3.5 years (Leigh et al., 2013).

The benefit of minocycline is that it is available by prescription currently and it can be used clinically, whereas the mGluR5 antagonists and the $GABA_A$ and $GABA_B$ agonists described above are not currently available clinically, although this may change in 2015. Minocycline is usually well tolerated, although it can cause GI upset, loose stools, skin sensitivity to the sun, and darkening of the skin, gums, or nails with prolonged use (Smith & Leyden 2005; Utari et al., 2010). Graying of the permanent teeth can also occur when used by children under age eight before the permanent teeth have emerged, so it is not FDA-approved for children under eight. Parents must decide whether the use of minocycline that can improve synaptic connections is worth the chance of gray teeth, although the latter problem can be fixed cosmetically with dental plating. In rare cases a lupus-like syndrome can develop, with a rash, swollen joints, or an autoimmune hepatitis, but this is reversible once the minocycline is stopped. Rarely, increased intracranial pressure can develop, leading to a severe headache called *pseudotumor cerebri*, so parents should be warned that if a rash, swollen joints, or persistent headache occur, the minocycline should be stopped.

Because minocycline is an antibiotic, it will change the flora in the GI tract, so it is recommended that a probiotic be utilized during treatment with minocycline. Minocycline and milk or milk products can chelate together, so milk should be avoided ½ to 1 hour before and after minocycline is given orally (Utari et al., 2010). We also recommend checking an ANA level every six months during minocycline treatment. Our preliminary data show that approximately 26% of children with FXS develop a positive antinuclear antibody (ANA) titer; however, 20% have a baseline positive titer (Rafika et al., unpublished data), similar to the autism population (20%, Mostafa & Kitchener, 2009) but higher than the 5% to 15% positive ANA in children in the general population (Wananukul et al., 2005).

Minocycline has been studied as a neuroprotective agent in diseases such as Huntington's disease and multiple sclerosis (Plane et al., 2010). There are several mechanisms by which minocycline has been theorized to exert its neuroprotective effects and anti-inflammatory effects, including inhibiting microglial activation, decreasing caspase activity, and through antiapoptotic properties (Plane et al., 2010; Figure 9.3). It is unclear whether these neurobiological effects may be beneficial for those with FXS and perhaps also for those with premutation developmental problems. For some

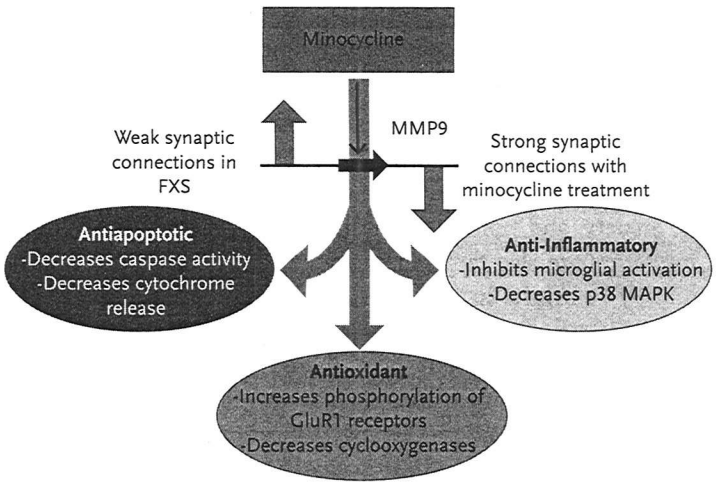

Figure 9.3:
Roles played by minocycline in neuroprotection and in lowering MMP9 levels to strengthen synaptic connections in those with FXS. Figure art developed by Dr. Paul Hagerman and Paul Doucet.

children with the premutation, there is a lowering of FMRP levels, particularly in the upper premutation range, which in turn would increase MMP9 levels. Therefore, those with the premutation and developmental problems related to lowered FMRP levels are predicted to improve on minocycline. This has been seen on a clinical basis in a handful of children, but controlled studies have not been carried out in premutation carriers. It is also possible that the immune-mediated problems that are experienced by some adult premutation carriers, such as fibromyalgia or multiple sclerosis, may also improve with minocycline. The neuroprotective and antiapoptotic effects of minocycline may also be beneficial for FXTAS, but so far these studies have not been carried out.

ADDITIONAL CONSIDERATIONS FOR TREATMENT

While the medications listed above are the focus of currently available targeted treatments, additional compounds have promising mechanisms for treatment in FXS. A recent open-label study of lithium in patients with FXS showed improvements in social behavior according to the ABC-C total score, VAS, Vineland-II maladaptive behavior subscale, and Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) list learning (Berry-Kravis et al., 2008) (Figure 9.2). Only mild side effects were seen,

although seven subjects experienced polyuria/polydipsia (Berry-Kravis et al., 2008). P21-activated kinase (PAK) inhibitors are another targeted treatment under development for FXS. Hayashi et al. (2007) demonstrated biological and behavioral phenotypical rescue in the *fmr1*-KO mouse, including partial restoration of irregular spine density and shape in the forebrain as well as partial and full amelioration of locomotor activity, stereotypy, anxiety, and trace fear conditioning with the *dnPAK* transgene. PI3K inhibitors are also targeted treatments in the *fmr1*-KO mouse and will probably be helpful in human studies when they are initiated (Gross et al., 2010).

The most robust treatment plan should be multifaceted, including non-pharmaceutical interventions such as speech, language, and/or occupational sensory integration therapy; educational/behavioral interventions such as the use of Early Start Denver Model for ASD (Dawson et al., 2010) ; the use of digital technology such as the iPAD learning programs to enhance academic, language, and socialization skills; in combination with targeted treatments, symptom-focused medications, and a healthy diet including antioxidants. There is strong evidence for oxidative stress in neurons with either the premutation (Chen et al., 2010; Cao et al., 2012) or the full mutation (de Diego-Otero et al., 2008), and the use of antioxidants such as NAC (N-acetylcysteine), alpha tocopherol (vitamin E), or melatonin (Romero-Zerbo & Decara, 2009) has been shown to normalize synaptic connections in the *fmr1*-KO mouse (de Diego-Otero et al., 2008). We therefore recommend antioxidants in the diet of children or adults with the premutation or the full mutation.

This is an exciting time for the use of targeted treatments in those with FXS because of the possibility of reversing the ID and behavioral problems associated with this and other related disorders with similar neurobiological changes. The future looks bright if the funding can be marshaled to carry out the studies that will demonstrate efficacy in individuals with FXS.

DISCLOSURES

This work was supported by grant R40 MC 22641 from the Maternal and Child Health Research Program, Maternal and Child Health Bureau (Combating Autism Act of 2006, as amended by the Combating Autism Reauthorization Act of 2011); Health Resources and Services Administration, Department of Health and Human Services, National Institutes of Health grants HD036071, HD02274, AG032119, and AG032115; DOD PR101054; support from the Health and Human Services Administration on Developmental Disabilities grant 90DD05969; and the National Center for Advancing Translational Research UL1 TR000002.

Corresponding author—Dr. Randi J. Hagerman, MIND Institute, UC Davis Health System, 2825 50th Street, Sacramento, CA 95817; email: randi.hagerman@ucdmc.ucdavis.edu; telephone: (916) 703-0247.

REFERENCES

Acosta, M. T., Kardel, P. G., Walsh, K. S., Rosenbaum, K. N., Gioia, G. A., & Packer, R. J. (2011). Lovastatin as treatment for neurocognitive deficits in neurofibromatosis type 1: Phase I study. *Pediatric neurology*, *45*(4), 241–245.

Au, J., Akins, R., Berkowitz-Sutherland, L., Tang, H., Chen, Y., Boyd, A., et al. (2013). Prevalence and risk of migraine headaches in adult fragile X premutation carriers. *Clinical genetics*, *84*(6), 846–551. doi: 10.1111/cge.12109

Bailey, D. B. Jr, Raspa, M., Bishop, E., & Holliday, D. (2009). No change in the age of diagnosis for FXS: Findings from a national parent survey. *Pediatrics*, *142*, 527–533.

Bagni, C., Tassone, F., Neri, G., & Hagerman, R. (2012). Fragile X syndrome: Causes, diagnosis, mechanisms, and therapeutics. *Journal of clinical investigation*, *122*(12), 4314.

Bear, M. F., Huber, K. M., & Warren, S. T. (2004). The mGluR theory of fragile X mental retardation. *Trends in neurosciences*, *27*, 370–377.

Berry-Kravis, E., Sumis, A., Hervey, C., Nelson, M., Porges, S. W., Weng, N., et al. (2008). Open-label treatment trial of lithium to target the underlying defect in fragile X syndrome. *Journal of developmental & behavioral pediatrics*, *29*(4), 293–302

Berry-Kravis, E., Hessl, D., Coffey, S., Hervey, C., Schneider, A., Yuhas, J., et al. (2009). A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *Journal of medical genetics*, *46*(4), 266–271.

Berry-Kravis, E., Knox, A. & Hervey, C. (2011). Targeted treatments for fragile X syndrome. *Journal of neurodevelopmental Disorders*, *3*, 193–210.

Berry-Kravis, E. M., Hessl, D., Rathmell, B., Zarevics, P., Cherubini, M., Walton-Bowen, K., et al. (2012). Effects of STX209 (arbaclofen) on neurobehavioral function in children and adults with fragile X syndrome: a randomized, controlled, phase 2 trial. *Science and translational medicine*, *4*, 152ra127.

Bilousova, T. V., Dansie, L., Ngo, M., Aye, J., Charles, J. R., Ethell, D. W., & Ethell, I. M. (2009). Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. *Journal of medical genetics*, *46*(2), 94–102.

Bourgeois, J. A., Seritan, A. L., Melina Casillas, E., Hessl, D., Schneider, A., Yang, Y., et al. (2011). Lifetime prevalence of mood and anxiety disorders in fragile X premutation carriers. *Journal of clinical psychiatry*, *72*(2), 175.

Bretherick, K. L., Fluker, M. R., & Robinson, W. P. (2005). FMR1 repeat sizes in the gray zone and high end of the normal range are associated with premature ovarian failure. *Human genetics*, *117*(4), 376–382.

Buonocore, G., Perrone, S., Turrisi, G., Kramer, B. W., & Balduini, W. (2012). New pharmacological approaches in infants with hypoxic-ischemic encephalopathy. *Current pharmaceutical design*, *18*(21), 3086–3100.

Cao, Z., Hulsizer, S., Tassone, F., Tang, H. T., Hagerman, R. J., Rogawski, M. A., et al. (2012). Clustered burst firing in *FMR1* premutation hippocampal neurons: Amelioration with allopregnanolone. *Human Molecular Genetics*, *21*, 2923–2935.

Chang, S. Bray, S. M., Li, Z., Zarnescu, D. C., He, C., Jin, P., Warren, S. T. (2008). Identification of small molecules rescuing fragile X syndrome phenotypes in Drosophila. *Nature chemical biology*, *4*, 256–263.

Chen, Y., Tassone, F., Berman, R. F., Hagerman, P. J., Hagerman, R. J., Willemsen, R., & Pessah, I. N. (2010). Murine hippocampal neurons expressing Fmr1 gene premutations show early developmental deficits and late degeneration. *Human molecular genetics*, *19*(1), 196–208.

Chonchaiya, W., Au, J., Schneider A., et al. (2012). Increased prevalence of seizures in boys who were probands with the *FMR1* premutation and co-morbid autism spectrum disorder. *Human genetics*, *131*(4), 581–589.

Cornish, K., Cole, V., Longhi, E., Karmiloff-Smith, A., & Scerif, G. (2013). Mapping developmental trajectories of attention and working memory in fragile X syndrome: Developmental freeze or developmental change? *Development and psychopathology*, *25*(02), 365–376.

Cordeiro, L., Ballinger, E., Hagerman, R., & Hessl, D. (2011). Clinical assessment of DSM-IV anxiety disorders in fragile X syndrome: prevalence and characterization. *Journal of neurodevelopmental disorders*, *3*(1), 57–67.

Crawford, D. C., Acuña, J. M., & Sherman, S. L. (2001). *FMR1* and the fragile X syndrome: human genome epidemiology review. *Genetics in medicine*, *3*(5), 359–371.

Curia, G., Papouin, T., Séguéla, P., & Avoli, M. (2009). Down-regulation of tonic GABAergic inhibition in a mouse model of fragile X syndrome. *Cerebral cortex*, *19*(7), 1515–1520.

D'Hulst, C., De Geest, N. et al. (2006). Decreased expression of the GABAA receptor in fragile X syndrome. *Brain research*, *1121*, 238–245.

Darnell, J. C., Van Driesche, S. J., Zhang, C., Hung, K. Y. S., Mele, A., Fraser, C. E., et al. (2011). FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell*, *146*(2), 247–261.

Darnell, J. C., & Klann, E. (2013). The translation of translational control by FMRP: therapeutic targets for FXS. *Nature neuroscience*, *16*(11), 1530–1536. doi: 10.1038/nn.3379

Dawson, G., Rogers, S., Munson, J., Smith, M., Winter, J., Greenson, J., et al. (2010). Randomized, controlled trial of an intervention for toddlers with autism: the Early Start Denver Model. *Pediatrics*, *125*(1), e17–e23.

de Diego-Otero, Y., Romero-Zerbo, Y., el Bekay, R., Decara, J., Sanchez, L., Rodriguez-de Fonseca, F., & del Arco-Herrera, I. (2008). α-tocopherol protects against oxidative stress in the fragile X knockout mouse: An experimental therapeutic approach for the Fmr1 deficiency. *Neuropsychopharmacology*, *34*(4), 1011–1026.

Dolen, G., Osterweil, E., Rao, B. S., Smith, G. B., Auerbach, B. D., Chattarji, S., Bear, M. F. (2007). Correction of fragile X syndrome in mice. *Neuron*, *56*, 955–962.

Dölen, G., Carpenter, R. L., Ocain, T. D., & Bear, M. F. (2010). Mechanism-based approaches to treating fragile X. *Pharmacology & therapeutics*, *127*(1), 78–93.

Erickson, C. A., Mullett, J. E., & McDougle, C. J. (2010). Brief report: acamprosate in fragile X syndrome. *Journal of autism and developmental disorders*, *40*(11), 1412–1416.

Erickson, C. A., Stigler, K. A., Wink, L. K., Mullett, J. E., Kohn, A., Posey, D. J., & McDougle, C. J. (2011). A prospective open-label study of aripiprazole in fragile X syndrome. *Psychopharmacology*, *216*(1), 85–90.

El Idrissi, A., Boukarrou, L., Dokin, C., & Brown, W. T. (2009). Taurine improves congestive functions in a mouse model of fragile X syndrome. *Taurine* 7, 191–198.

Farzin, F., Perry, H., Hessl, D., Loesch, D., Cohen, J., Bacalman, S., et al. (2006). Autism spectrum disorders and attention-deficit/hyperactivity disorder in boys with the fragile X premutation. *Journal of developmental & behavioral pediatrics*, *27*(2), S137–S144.

Fatemi, S. H., Kneeland, R. E., Liesch, S. B., & Folsom, T. D. (2010). Fragile X mental retardation protein levels are decreased in major psychiatric disorders. *Schizophrenia research*, *124*(1–3), 246.

Fatemi, S. H., Folsom, T. D., Kneeland, R. E., & Liesch, S. B. (2011). Metabotropic glutamate receptor 5 upregulation in children with autism is associated with underexpression of both fragile X mental retardation protein and GABAA receptor beta 3 in adults with autism. *The Anatomical record: advances in integrative anatomy and evolutionary biology*, *294*(10), 1635–1645.

Goodlin-Jones, B. L., Tassone, F., Gane, L. W., & Hagerman, R. J. (2004). Autistic spectrum disorder and the fragile X premutation. *Journal of developmental & behavioral pediatrics*, *25*(6), 392.

Gross, C., Nakamoto, M., Yao, X., Chan, C. B., Yim, S. Y., Ye, K., Warren, S. T., & Bassell, G. J. (2010). Excess phosphoinositide 3-kinase subunit synthesis and activity as a novel therapeutic target in fragile X syndrome. *Journal of neuroscience*, *30*, 10624–10638.

Hamlin, A. A., Sukharev, D., Campos, L., Mu, Y., Tassone, F., Hessl, D., et al. (2012). Hypertension in *FMR1* premutation males with and without fragile X-associated tremor/ataxia syndrome (FXTAS). *American journal of medical genetics part a*, *158A*, 1304–1309.

Hagerman, R. J., & Hagerman, P. J. (Eds.). (2002). *Fragile X syndrome: Diagnosis, treatment, and research*. Baltimore, MD: Johns Hopkins University Press.

Hagerman, R. J., Berry-Kravis, E., Kaufmann, W. E., Ono, M. Y., Tartaglia, N., Lachiewicz, A., & Tranfaglia, M. (2009). Advances in the treatment of fragile X syndrome. *Pediatrics*, *123*(1), 378–390.

Hagerman, R. J., Lauterborn, J., Au, J., & Berry-Kravis, E. (2012). Fragile X syndrome and targeted treatment trials. *Results and problems in cell differentiation*, *54*, 297–335.

Hagerman, R. J. & Hagerman, P. (2013). Advances in the clinical and molecular research regarding the premutation including FXTAS: The other side of fragile X. *Lancet neurology*, *12*, 786–798.

Hall, D. A., Berry-Kravis, E., Zhang, W., Tassone, F., Spector, E., Zerbe, G., et al. (2011). *FMR1* gray zone alleles: Association with Parkinson's disease in women. *Movement disorders*, *26*(10), 1900–1906.

Harris, S. W., Hessl, D., Goodlin-Jones, B., Ferranti, J., Bacalman, S., Barbato, I., et al. (2008). Autism profiles of males with fragile X syndrome. *American journal on mental retardation*, *113*, 427–438.

Hayashi, M. L., Rao, B. S., Seo, J. S., Choi, H. S., Dolan, B. M., Choi, S. Y., et al. (2007). Inhibition of p21-activated kinase rescues symptoms of fragile X syndrome in mice. *Proceedings of the national academy of sciences*, *104*(27), 11489–11494.

Henderson, C., Wijetunge, L., Kinoshita, M. N., Shumway, M., Hammond, R. S., Postma, F. R., et al. (2012). Reversal of disease-related pathologies in the fragile X mouse model by selective activation of GABAB receptors with arbaclofen. *Science and translational medicine*, *4*, 152ra128.

Heulens, I., D'Hulst, C., Van Dam, D., De Deyn, P. P., & Kooy, R. F. (2012). Pharmacological treatment of fragile X syndrome with GABAergic drugs in a knockout mouse model. *Behavioural brain research*, *229*(1), 244–249.

Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., et al. (2012). De novo gene disruptions in children on the autistic spectrum. *Neuron*, *74*(2), 285–299.

Jacquemont, S., Curie, A., Des Portes, V., Torrioli, M. G., Berry-Kravis, E., Hagerman, R. J., et al. (2011). Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. *Science translational medicine*, *3*(64), 64ra1–64ra1.

Kaufmann, W. E., Cortell, R., Kau, A., Bukelis, I., Tierney, E., Gray, R., et al. (2004). Autism spectrum disorder in FXS: communication, social interaction, and specific behaviors. *American journal of medical Genetics part a*, *129A*, 225–234.

Kim, S. Y., Burris, J., Bassal, F., Koldewyn, K., Chattarji, S., Tassone, F., et al. (2014). Fear-specific amygdala function in children and adolescents on the fragile X spectrum: a dosage response of the *FMR1* gene. *Cerebral cortex*, *24*(3), 600–613. doi: 10.1093/cercor/bhs341

Kovács, T., Kelemen, O., & Kéri, S. (2013). Decreased fragile X mental retardation protein (FMRP) is associated with lower IQ and earlier illness onset in patients with schizophrenia. *Psychiatry research*, *210*(3), 690–693. doi: 10.1016/j.psychres.2012.12.022

Leigh, M. J. S., Nguyen, D. V., Mu, Y., Winarni, T. I., Schneider, A., Chechi, T., et al. (2013). A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile X syndrome. *Journal of developmental & behavioral pediatrics*, *34*(3), 147–155.

Li, M., & Losordo, D. W. (2007). Statins and the endothelium. *Vascular pharmacology*, *46*(1), 1–9.

Liu, Y., Winarni, T. I., Zhang, L., Tassone, F., & Hagerman, R. J. (2012). Fragile X-associated tremor/ataxia syndrome (FXTAS) in grey zone carriers. *Clinical genetics*, *84*(1), 74–77. doi: 10.1111/cge.12026.

Loesch, D. Z., Huggins, R. M., & Hagerman, R. J. (2004). Phenotypic variation and FMRP levels in fragile X. *Mental retardation and developmental disabilities research reviews*, *10*(1), 31–41.

Loesch, D. Z., Bui, Q. M., Huggins, R. M., Mitchell, R. J., Hagerman, R. J., & Tassone, F. (2007). Transcript levels of the intermediate size or grey zone fragile X mental retardation 1 alleles are raised, and correlate with the number of CGG repeats. *Journal of medical genetics*, *44*(3), 200–204.

Massey, P. V., & Bashir, Z. I. (2007). Long-term depression: Multiple forms and implications for brain function. *Trends in neurosciences*, *30*, 176–184.

Meredith, R. M., De Jong, R., Mansvelder, H. D. (2011). Functional rescue of excitatory synaptic transmission in the developing hippocampus in Fmr1-KO mouse. *Neurobiology of disease*, *41*, 104e110.

Michalon, A., Sidorov, M., Ballard, T. M., Ozmen, L., Spooren, W., Wettstein, J. G., et al. (2012). Chronic pharmacological mGlu5 inhibition corrects fragile X in adult mice. *Neuron*, *74*, 49e56.

Michaluk, P., Wawrzyniak, M., Alot, P., Szczot, M., Wyrembek, P., Mercik, K., et al. (2011). Influence of matrix metalloproteinase MMP-9 on dendritic spine morphology. *Journal of cell science*, *124*(19), 3369–3380.

Mostafa, G. A., & Kitchener, N. (2009). Serum anti-nuclear antibodies as a marker of autoimmunity in Egyptian autistic children. *Pediatric neurology*, *40*(2), 107–112.

Nakamoto, M., Nalavadi, V., Epstein, M. P., Narayanan, U., Bassell, G. J., Warren, S. T. (2007). Fragile X mental retardation protein deficiency leads to excessive mGluR5-dependent internalization of AMPA receptors. *Proceedings of the national academy of sciences USA*, *104*, 15537–15542.

Olmos-Serrano, J. L., Paluszkiewicz, S. M., Martin, B. S., Kaufmann, W. E., Corbin, J. G., & Huntsman, M. M. (2010). Defective GABAergic neurotransmission and pharmacological rescue of neuronal hyperexcitability in the amygdala in a mouse model of fragile X syndrome. *Journal of neuroscience*, *30*, 9929–9938.

Osterweil, E. K., Krueger, D. D., Reinhold, K., & Bear, M. F. (2010). Hypersensitivity to mGluR5 and ERK1/2 leads to excessive protein synthesis in the hippocampus of a mouse model of fragile X syndrome. *Journal of neuroscience*, *30*(46), 15616–15627.

Osterweil, E. K., Chuang, S.-C., Chubykin, A. A., Sidorov, M., Bianchi, R., Wong, R. K., & Bear, M. F. (2013). Lovastatin corrects excess protein synthesis and prevents epileptogenesis in a mouse model of fragile X syndrome. *Neuron*, *77*(2), 243–250.

Paribello, C., Tao, L., Folino, A., Berry-Kravis, E., Tranfaglia, M., Ethell, I., & Ethell, D. (2010). Open-label add-on treatment trial of minocycline in fragile X syndrome. *BMC Neurology*, *10*(1), 91.

Plane, J. M., Shen, Y., Pleasure, D. E., & Deng, W. (2010). Prospects for minocycline neuroprotection. *Archives of neurology*, *67*(12), 1442.

Pin, J. P., & Prézeau, L. (2007). Allosteric modulators of GABAB receptors: mechanism of action and therapeutic perspective. *Current neuropharmacology*, *5*(3), 195.

Prata, M. M., Nogueira, A. C., Pinto, J. R., Correia, A. M., Vicente, O., Rodrigues, M. C., & Miguel, M. J. (1994). Long-term effect of lovastatin on lipoprotein profile in patients with primary nephrotic syndrome. *Clinical nephrology*, *41*(5), 277.

Romero-Zerbo, Y., Decara, J., et al. (2009). Protective effects of melatonin against oxidative stress in Fmr1 knockout mice: a therapeutic research model for the fragile X syndrome. *Journal of pineal research*, *46*(2), 224–234.

Rotschafer, S. E., Trujillo, M. S., Dansie, L. E., Ethell, I. M., & Razak, K. A. (2011). Minocycline treatment reverses ultrasonic vocalization production deficit in a mouse model of Fragile X Syndrome. *Brain research*, *1439*, 7–14.

Sansone, S. M., Widaman, K. F., Hall, S. S., Reiss, A. L., Lightbody, A., Kaufmann, W. E., et al. (2012). Psychometric study of the aberrant behavior checklist in Fragile X syndrome and implications for targeted treatment. *Journal of autism and developmental disorders*, *42*(7), 1377–1392.

Siller, S. S., & Broadie, K. (2011). Neural circuit architecture defects in a Drosophila model of fragile X syndrome are alleviated by minocycline treatment and genetic removal of matrix metalloproteinase. *Disease models & mechanisms*, *4*(5), 673–685.

Smith, K., & Leyden, J. J. (2005). Safety of doxycycline and minocycline: a systematic review. *Clinical therapeutics*, *27*(9), 1329–1342.

Sullivan, S. D., Welt, C., & Sherman, S. (2011). *FMR1* and the continuum of primary ovarian insufficiency. *Seminars in Reproductive Medicine*, *29*, 299–307.

Tassone, F., Hagerman, R. J., Taylor, A. K., Gane, L. W., Godfrey, T. E., & Hagerman, P. J. (2000). Elevated levels of *FMR1* mRNA in carrier males: A new mechanism of involvement in the fragile-X syndrome. *American journal of human genetics*, *66*(1), 6–15.

Tassone, F., Long, K. P., Tong, T., Lo, J., Gane, L., Berry-Kravis, E., et al. (2012). *FMR1* CGG allele size and prevalence ascertained through newborn screening in the United States. *Genome medicine*, *4*, 100. doi:10.1186/gm401

Thomas, A. M., Bui, N., Perkins, J. R., Yuva-Paylor, L. A., & Paylor, R. (2012). Group I metabotropic glutamate receptor antagonists alter select behaviors in a mouse model for fragile X syndrome. *Psychopharmacology (Berlin)*, *219*, 47e58.

Utari, A., Chonchaiya, W., Rivera, S. M., Schneider, A., Hagerman, R. J., Faradz, S. M., et al. (2010). Side effects of minocycline treatment in patients with fragile X syndrome and exploration of outcome measures. *American journal on intellectual and developmental disabilities*, *115*(5), 433–443.

Wananukul, S., Voramethkul, W., Kaewopas, Y., Hanvivatvong, O. (2005). Prevalence of positive antinuclear antibodies in healthy children. *Asian pacific journal of allergy immunology*, *23*, 153–157.

Watson, D., Hoeft, F., Garrett, A. S., Hall, S. S., & Reiss, A. L. (2009). Aberrant brain activation during gaze processing in boys with fragile X syndrome. *Archives of general psychiatry*, *65*, 1315–1323.

Winarni, T. I., Schneider, A., Borodyanskara, M., & Hagerman, R. J. (2012a). Early intervention combined with targeted treatment promotes cognitive and behavioral improvements in young children with fragile X syndrome. *Case Reports in Genetics*, *2012*, 280813:1–4. doi: 10.1155/2012/280813

Winari, T. I., Chonchaiya, W., Adams, E., Au, J., Yi Mu, Rivera, S. M., Nguyen, D. V., Hagerman, R. J. (2012b). Sertraline may improve language developmental trajectory in young children with fragile X syndrome: a retrospective chart review. *Autism Research and Treatment*, 2012; Epub 2012 May 31. doi:10.1155/2012/104317.

Wirojanan, J., Jacquemont, S., Diaz, R., Bacalman, S., Anders, T. F., Hagerman, R. J., & Goodlin-Jones, B. L. (2009). The efficacy of melatonin for sleep problems in children with autism, fragile X syndrome, or autism and fragile X syndrome. *Journal of Clinical Sleep Medicine*, *5*(2), 145–150.